Quantitative Analysis of Human Chronotypes

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In Memoriam

Dr. Klaus Pasold

(1947-2004)

1. Introduction

1. Introduction

1.1. Rhythmicity, a general quality of life

The central issue of evolution is the selection of randomly occuring genetic modifications (mutations) beneficial for the adaptation to environmental conditions that define ecological niches (Darwin 1859). While spatial niches (biotopes) consist of different geological and biological structures (e.g. rain forest, desert, mountains, deep sea,…) temporal niches (chronotopes) comprise time structures like day and night, seasons, and moon phases. (Roenneberg 1992). Adaptation to spatial niches is reflected by an organisms morphology (e.g. lungs vs. gills) that does not change in general throughout life because spatial niches are usually not left. Temporal niches in contrast undergo various repeating changes caused by i) earths rotation around the sun, ii) earths rotation around its own axis, and iii) the moons rotation around earth. Thus, as soon life became dependent on conditions affected by one or more of these astronomical phenomenons (light, temperature, humidity,…) it had to cope with rhythmic changes in order to use opportune ranges of these conditions as well as to avoid the unfavourable ones.

Rhythmically changing environmental conditions are challenges for every organism. Therefore, it is important to be prepaired for conditions either advantageous or disadvantageous rather than just reacting to them. For any organism that is prey of another it is vitally important to know at which time of the day predators are most likely around. The same reasons apply for animals inhabiting the beach in order to know when the tide is low or high. Fured animals need their winter fur before temperature starts to decrease drastically in autumn and the photosynthetic machinery of plants must be fully working by the first rays of sunlight. Biological clocks - reflecting rhythms caused by the interplay of sun, moon, and earth - make it possible to anticipate forthcoming changes in the environment.

Rhythmicity can be found in all organisms, ranging from production/degradation of molecules to fluctuations within and between whole populations. Different classes of rhythms have been defined: rhythms with periods shorter than one day are called ultradian (for review see Gerkema, 2002), those with periods longer than 24h are infradian rhythms, analogous to the wave lengths of light. **Fig.1.1.** shows some examples of mammalian rhythms with respect to this division.

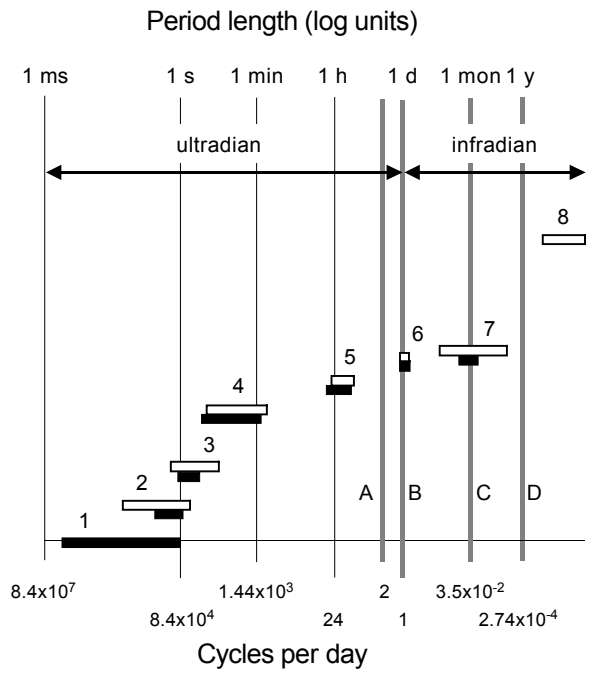


Fig.1.1: Ranges of different rhythms for humans (black bars) and mammals (white bars).
1: Processes of the central nervous system
2: Heart rate
3: Respiration rate
4: Circulation, blood pressure, biochemical processes
5: Activity bursts, REM sleep phases, hormone secretion
6: Circadian rhythms (see text)
7: Ovarian cycle
8: Population rhythms

A-D: The four "circa" rhythms (see text):
A: Tidal rhythms (circatidal)
B: Daily rhythms (circadian)
C: Monthly rhythms (circalunar)
D: Annual rhythms (circannual)
(redrawn from Aschoff, 1981 & Roenneberg, 1998)

1.2. The four circa rhythms

Rhythms can either be purely driven, which means that they do not exist without an exogenous pacemaking signal. Some rhythms however keep on cycling, without being stimulated, with a period close to that of the stimulus. Because the period of these so called free running endogenous rhythms is only about the period of the exogenous signal, they are called circa- (=about) rhythms. This terminology has first been proposed by Halberg in1959 (**Fig.1.1**) for daily rhythms, however, it is applied for all rhythms with environmental counterpart.

1.2.1. Circannual rhythms

Most animals and plants display physiological changes throughout the year. Circannual rhythmicity has been demonstrated in plants and many animal species like mammals, birds, reptiles, arthropodes, and mollusces comprising functions like hibernation, migratory behaviour, locomotor activity, and hormonal status (Gwinner, 1981). The most obvious phenomenons are blossom of plants and reproduction of animals. But also molt, migration, hibernation, ... are under control of an endogenous clock (Gwinner 1986; Aschoff, 1981). The most important environmental influences on annual rhythmicity are photoperiod (ratio of day and night length) and temperature. Some mammals become reproductive with increasing day length (long-day breeders, e.g. many rodents) while others are reproductive during the short-day period of the year (short-day breeders, e.g. sheep). The state of reproductiveness is

most probably mediated by melatonin secretion which encodes day length information (Goldman, 2001; Wehr 2001). In birds, molt appears every year even when photoperiod and temperature are kept constant, also testicular width follows a cirannual rhythm. Both exhibit a period shorter than one year (Gwinner, 2003). Hibernation of ground sqirrels occurs about once a year when animals are kept in conditions like constant darkness (DD), constant light (LL) or in a constant cycle of 12h of light and 12h of darkness (LD 12:12). Again, rhythmicity observed was shorter than 12 months. In general, persisting circannual rhythms can vary greatly, ranging from 7 to 15 months, depending on species. But even within individual species, considerable variation can be observed (Gwinner, 1981).

Circannual investigations under constant conditions are, of course, not applicable for man but various human behaviour is documented well over decades or even centuries and statistical analysis is the approach of choice for investigating human circannual rhythmicity. A landmark study has been performed by Roenneberg and Aschoff on the annual rhythm of human reproduction analyzing monthly conception rates of more than 150 worldwide regions. They could show that rhythms of conception rates follow a characteristic waveform depending on geographical region and that rhythms are stable over a long time. Rhythms on the Northern and Southern Hemisphere resemble each other with a phase shift of six months. However, phase and amplitude changed during the last century (Roenneberg & Aschoff, 1990a). The amplitude of birth rates (percentage of deviation from annual mean) in Spain, e.g., drastically decreased after an industrialisation campaign in the 1960s which created more indoor working places leading to a change of light exposure. (Roenneberg *et al*, 2004; Roenneberg 2004). Furthermore, a dependency of human conception rates on photoperiod and temperature could be shown. These results are different for different regions of the world but still are highly systematic, depending on latitude and on temperature differences between summer and winter. As a concomitant of industrialization, an increased independence from photoperiod and temperature led to a deseasonalisation of conception rhythms (Roenneberg & Aschoff, 1990b). For references on other human habits following a seasonal variation see Roenneberg (1998).

1.2.2. Circatidal and circalunar rhythms

Tidal changes are generated by gravitational and centrifugal forces resulting from the interplay of earth, moon, and sun. Most species affected are of marine origin but also many terrestial species inhabit tidal regions and have to deal with high and low water levels. Endogenous tidal rhythms can be observed by many species, however, in most cases, rhythms damp out within several cycles. Nevertheless, some species exhibit robust rhythms over several weeks (for references and details see Neumann, 1981).

Adaptation to moon phases can be observed for twighlight and night active animals although these rhythms are in most cases merely passive reactions to the amount of light changing from new moon to full moon. Some lunar rhythms, however, keep on cycling under constant conditions, e.g. the pit-building behaviour of the ant lion *Myrmeleon obscurus* (Youthed & Moran, 1969, cited in Neumann, 1981).

There are indications that the female menstruation cycle synchronizes with moon phases but this is rather a synchronisation to social or olfactory stimuli than caused by an endogenous circalunar clock (Roenneberg, 1998).

1.2.3. Circadian rhythms

Circadian rhythms are the most extensively investigated biological rhythms because almost all organisms, cyanobacteria, funghi, plants, and animals, including humans, are affected by the daily change of light and darkness. All further introduction will, therefore, focus on circadian rhythmicity.

1.3. Biological oscillators

1.3.1. Clocks and zeitgeber

Oscillating systems occur in a large variety throughout almost all organisms (Dunlap, 1999). Some rhythms, however, keep on cycling even in the absence of a pacemaking stimulus with a self-sustained endogenous rhythmicity. Internal clocks allow an organism the anticipation of environmental changes (see 1.1.) and are synchronized by rhythmically occuring environmental stimuli (zeitgeber, e.g. daily change of light and darkness). This synchronisation of endogenous biological clocks, however, is not just a response to the exogenous stimuli (driveness) but a systematic process (entrainment) that depends on many different properties of the clock and the exogenous stimuli (Roenneberg *et al*, 2003b). Biological clocks are, in principle, comparable to mechanical oscillators (in order to simplify the story, the term ***clock*** will be used synonymously with *biological and mechanical oscillators* and the term ***zeitgeber***, a German word that wonderfully confused not-German speaking students for decades and literally translated means *time giver*, is used instead of exogenous stimulus). With knowledge of the parameters summarized in **Fig.1.2.** the outcome of an entrained system (containing clock and zeitgeber) is predictable.

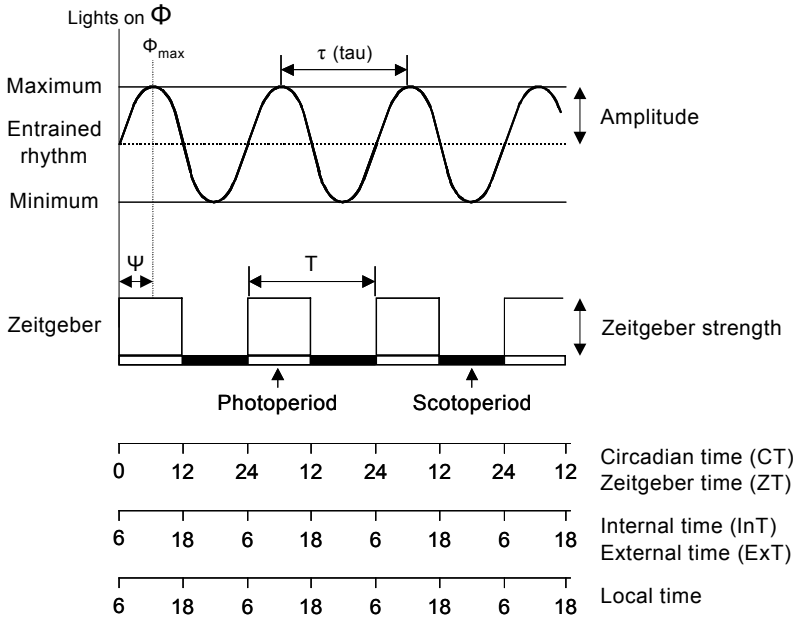


Fig.1.2.: Parameters of an entrainable clock (represented by an idealized sine curve) and the entraining stimulus (rectangles). Revised from Roenneberg, 1998.

Glossary:

τ (tau)	endogenous period of the clock
T	period of the zeitgeber cycle
Φ	phase marker
Φ	onset of zeitgeber
Ψ	phase angle of Φ

The terms Circadian time (CT) and Internal time (InT) refer to a clock cycling without entraining stimulus (free run constant conditions). Zeitgeber time (ZT) and External time (ExT) are applied to entrained systems. InT and ExT have been suggested by Daan *et al*, 2002 in order to simplify current chronobiological conventions. Local time is regular clock time.
For descriptions see text.

In the absence of any entraining zeitgeber, the self sustained clock "runs free", i.e., it cycles with its own inherent, endogenous period τ (tau). If a clock is entrained, the free running period adjusts to the period T of the entraining zeitgeber (τ=T). For example, most humans have a free running sleep-wake rhythm a little longer than 24 hours. This has first been shown for humans by Jürgen Aschoff with his legendary bunker experiments (Aschoff, 1965) and will be described in detail later.

If the clock was just synchronized it would be reset each cycle, independent of its endogenous period. Entrainment, in contrast, is a process that systematically functions depending on a) the period, b) the duration, and c) the strength of the entraining zeitgeber, furthermore, d) period and amplitude and e) the responsiveness of the clock (Roenneberg *et al*, 2005). These different parameters can be summarized with a phase response curve.

1.3.2. Entrainment

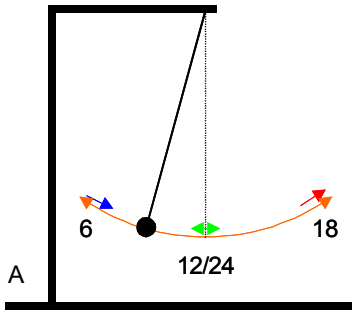
1.3.2.1 The two models of entrainment

In order to adjust an oscillator to a rhythmic zeitgeber, the strength of the zeitgeber and the phase of the endogenous clock cycle are of major importance. This somehow mixes up the two basic models of entrainment. The *continuous model* proposes that the intensity of light, as the most important zeitgeber in nature, is mainly responsible for changes in phase angle and free running period (see **Fig.1.2.**) which leads to an entrained rhythm. The model mainly bases on the observation that free running period under constant light conditions (LL) changes with increasing light intensity in many species. This observation is called Aschoff's

rule (for reference, see Menaker, 1971), and Jürgen Aschoff (1913 - 1998) was an advocate of this model. Another pioneer of chronobiology, Colin S. Pittendrigh (1918 - 1996), supported the *discrete model* which claims that individual entraining pulses are the basis for entrainment. Experiments with rodents showed that time point of application and not intensity of light is important to obtain stable phase angles (DeCoursey, 1972). For this model, only free running period and phase response curve (PRC) are important. In nature, a mix of both models might apply, depending on species and environmental conditions they are exposed to.

1.3.2.2. Phase response curve (PRC)

The phase shift caused by a zeitgeber depends on the Internal time (InT) at which the clock is hit. This is examplified by a simple model of a pendulum swinging with a given, self sustained amplitude and period (**Fig.1.3.A**)



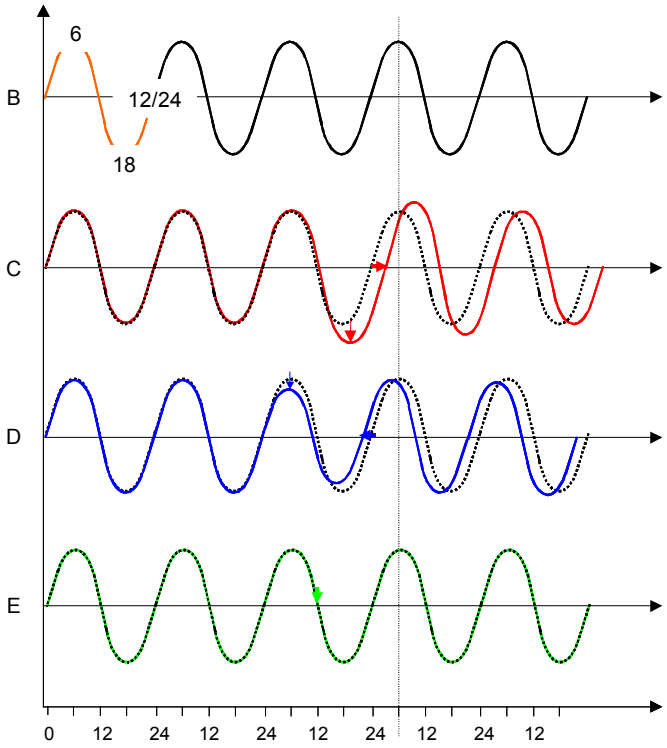


Fig.1.3.: A) simplified model of a clock (modified from Roenneberg *et al*, 2003). Orange arrowheads represent turning points at phase 6 and 18, respectively (1.3.B).

C) Phase delayed rhythm after a perturbation at phase 18

D) Phase advanced thythm after a perturbation at phase 6

E) Neither an advance nor a delay after a perturbation at phase 12

Dotted curves indicate non-disturbed rhythm. For details see text

The swing of the pendulum responds depending on the phase the perturbation is administrated. Giving a push with a certain intensity at phase 18 (red arrow, **Fig.1.3.A**) increases the amplitude (red horizontal arrow, **Fig.1.3.C**) and thus delays the turning point. After swinging back, every phase point is delayed (e.g. phase 24, red vertical arrow, **Fig.1.3.C**) and the rhythm is phase delayed. A push given at phase 6 (blue arrow, **Fig.1.3.A**) de-

creases the amplitude (blue horizontal arrow, **Fig.1.3.D**) and advances the turning point. This results in a phase advance of the rhythm (blue vertical arrow, **Fig.1.3.D**). At the point of maximum speed at phases 12 and 24 (green arrow, **Fig.1.3.A**), pushing the pendulum has no effect in shifting the rhythm (**Fig.1.3.D**). The response also depends on the strength of the perturbation. The stronger the push, the larger the phase shift. On the other hand, a perturbation can have no effect, regardless of the phase it is administrated, e.g., in our example, when the weight of the pendulum is very high (strong oscillator) and the strength of the push very low (weak zeitgeber). A push given with exactly the right strength and opposite to the direction of the pendulum at the right phase (12 or 24) can stop it from swinging or, if the push is even stronger, invert the rhythm and shift it over half a cycle (Roenneberg *et al*, 2003b).

In biological oscillators, the "dead zone" (phase 12 and 24, **Fig.1.3.A**) is usually much larger and can span over several hours. **Fig.1.4.A** shows an example of a phase response curve (PRC), originally from hamster (Daan & Pittendrigh, 1976a) but it will here be used for a hypothetical example of a day active animal.

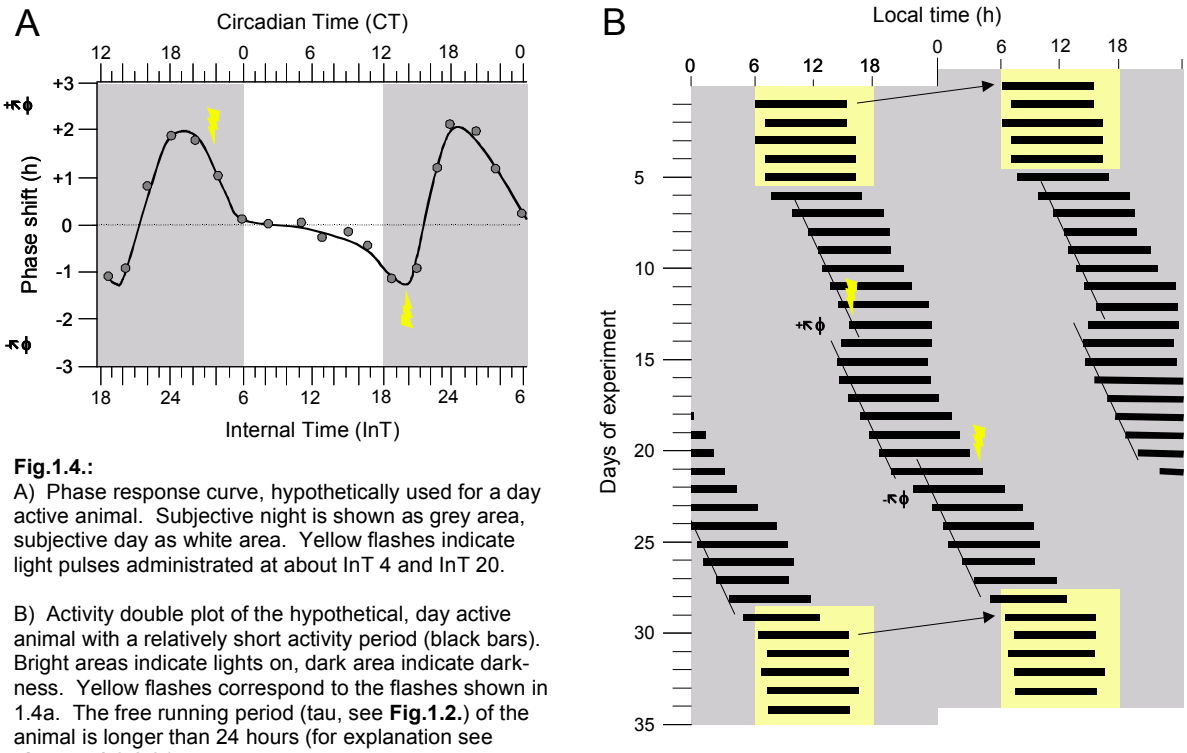


Fig.1.4.:
A) Phase response curve, hypothetically used for a day active animal. Subjective night is shown as grey area, subjective day as white area. Yellow flashes indicate light pulses administrated at about InT 4 and InT 20.

B) Activity double plot of the hypothetical, day active animal with a relatively short activity period (black bars). Bright areas indicate lights on, dark area indicate darkness. Yellow flashes correspond to the flashes shown in 1.4a. The free running period (tau, see **Fig.1.2.**) of the animal is longer than 24 hours (for explanation see **chapter 1.3.2.3.**).
Although the hypothetical animal could theoretically be a human this experiment is not applicable to man for ethical reasons.

Phase response curves (PRCs) are determined experimentally by first keeping the organism of investigation (e.g. mouse) under constant condition, e.g. complete darkness (DD). If, e.g., 240 mice are used for the experiment, 10 mice will receive a light pulse with a certain

duration and intensity, depending on experimantal questions, 1 hour after, e.g., onset of activity. The next 10 mice will receive the light pulse after 2 hour after onset of activity, … . The phase shift of onset of activity is measured for every group of mice and can be plotted as a phase response curve (**Fig.1.4.A**). In our example, the light pulse represented by the upper left flash in **Fig.1.4.A** results in a phase advance (positive deviation from the 0 line). The same flash is shown on **Fig.1.4.B** (upper left flash) and leads to an advance of the activity rhythm (black bars), corresponding to the phase shift shown in **Fig.1.3.D**. As shown in **Fig.1.4.B**, it takes some cycles until the activity rhythm shows again a stable free run, represented by regression lines drawn through onsets of activity. These delays are called transients and the number of cycles (duration of transients) it takes until a stable free run is achieved depends on the phase of the PRC at which the light pulse is administrated. Hitting the PRC at another phase results in a delay (**Fig.1.4.A+B**, lower right flashes and **Fig.1.3.C**) while light administrated between InT 6 to InT 12 (**Fig.1.4.A**) leads to no or only a very slight phase shift (**Fig.1.3.E**). On the other hand, even a self-sustained endogenous rhythm can give the impression of being purely driven when either the zeitgeber is very strong and/or the clock is very weak. In this case, the clock is, independent of actual phase position of the PRC, reset every time it is hit by the zeitgeber.

Another phenomenon of entrainment is masking. If mice are entrained to a light:dark cycle (LD) they are active during darkness and stop moving as soon as light is switched on. However, this is rather an acute response to light than an output of the entrained endogenous clock. To determine the "real" end of activity triggered by the clock, the mice are, after being entrained for several days, released into constant darkness (DD). The free running offset of activity can then be extrapolated back to the entrained days. A sophisticated approach of de-masking biological oscillators has recently been made by Roenneberg *et al* (2005). **Fig.1.5.** shows an example of masking for a hypothetical nocturnal animal.

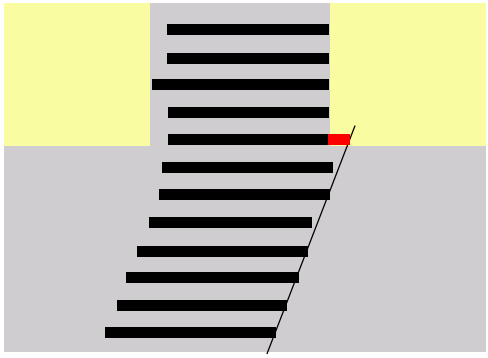


Fig.1.5.: Masking exemplified by an LD experiment with a nocturnal animal (bright area: light phase, grey area: dark phase, activity is shown as black bars). When lights are switched off the animal becomes active always at about the same time (ExT) every day. However, as soon as lights are switched on the animal stops moving. The real end of activity, controlled by the clock, is masked by the acute response to light. Some days after the animal has been released to DD it will establish a stable free running rhythms. The difference between the regression line drawn through the ends of activity of the stable free running rhythm and the end of activity of the last entrained day (red bar) indicates the duration of activity masked by light. In contrast to **Fig.1.4b**, the free running period (tau, see **Fig.1.2.**) here is shorter than 24 hours (for explanation see **chapter 1.3.2.3.**

All explanations above assumed that the PRC is not altered during a phase shift (phase-only system). This holds true for mechanical oscillator but is more complicated for biological oscillators and goes beyond the scope of this introduction. Reviews on entrainment are provided by Roenneberg *et al* (2003b) and Johnson *et al* (2003a+b).

1.3.2.3. Different clocks are entrained differently

Depending on free running period (FRP), different clocks have different phase angles (e.g. sleep onset or minimum body temperature) under the same entraining conditions. Different clocks under entrained and free running conditions are shown in **Fig.1.6.**

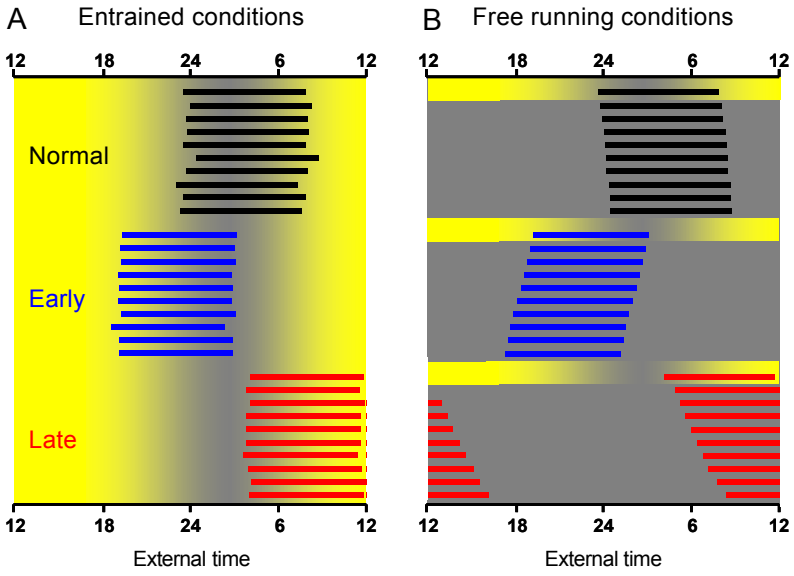


Fig.1.6.: Clocks with different free running periods (FRP). Bars represent sleep times (about 8 hours) of hypthetical humans for 10 days under entrained conditions (A: yellow - day, grey - night) and constant conditions (B: continuous grey area). In the right graph, subjects are released into constant conditions after the first day under entrained conditions.
Sleep times of a subject with a circadian clock with a FRP shorter than 24 hours is shown as blue bars (early type), a clock with a FRP more than 24 hours is shown with red bars (late type). A normal type is shown with black bars (FRP close to 24 hours). For details see text.

Most human circadian clocks exhibit FRPs slightly longer than 24 hours (black bars in **Fig.1.6.**). Even the clock of people working indoor all day can be synchronized to the 24 hour light/dark cycle. Short FRPs settle e.g. the daily sleep-wake-cycle earlier than long FRPs within the 24 hour day (sleep onset at about 20:00 in the evening for short FRPs and 4:00 in the morning for long FRPs). In other words, the stronger the zeitgeber (e.g. amount of outside light) the smaller the variance of the chronotype distribution. For a discussion on that issue see Roenneberg *et al* (2003a).

1.4. The mammalian circadian clock(s)

1.4.1. Localizing the central mammalian clock

A lot has already been known about circadian rhythms before the responsible circadian pacemaker (clock) has been located (Aschoff, 1960; Pittendrigh, 1960; Aschoff, 1965). In 1972, Moore & Eichler and Stephan & Zucker independently identified the suprachiasmatic nucleus (SCN) as the host of the endogenous mammalien circadian clock. The SCN consists of two bilaterally symmetrical nuclei in the anterior hypothalamus and each nucleus comprises about 10,000 neurons (Reppert & Weaver, 2001; Antle & Silver, 2005). Light reaches the SCN via the retinohypothalamic tract (RHC) which branches off the optical nerves. There are two ways of how incoming light is processed in the brain. The visual system is capable of high temporal and spatial resolution (image-forming retinal pathways). The circadian system integrates information about intensity, duration, and spectral composition of light, and thus, by predicting e.g. dusk and dawn, allows the orientation in the fourth dimension (non image-forming retinal pathways). Location of the SCN, light input and signal transduction are schematically shown in **Fig.1.7.**

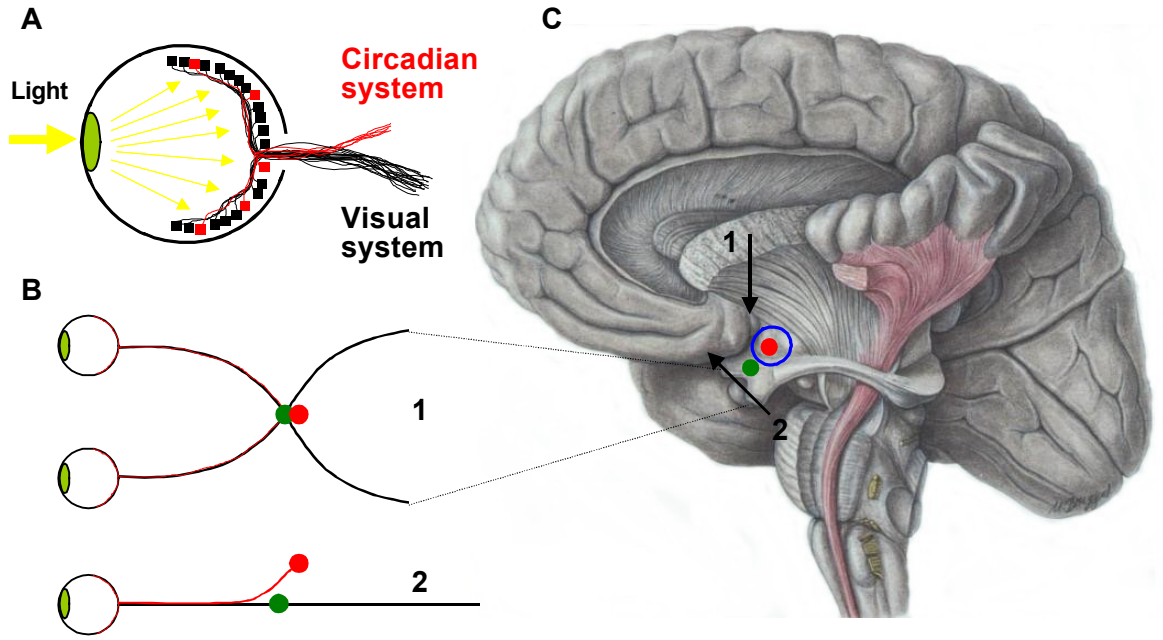


Fig.1.7.: Signal transduction of the circadian system (A+B) and location of the SCN (C).

A: Light falling into the eye is dispersed over the retina. Light information from the retina exits the eye via the optic nerve (black bundle). A sub population of retinal ganglion cells (red squares) are directly light sensitive and project to the SCN via the retinohypothalamic tract (RHT, red bundle). For details see text in **chapter 1.4.2.**

B: The optic nerves cross each other at the optic chiasm (gree dots). The suprachiasmatic nucleus (SCN) is located above (supra) the chiasm within the hypothalamus (red dots). The upper picture shows a view from above (arrow 1 in C), the lower picture shows a view from the side (arrow 2 in C). Photosensitive retinal ganglion cells and the RHT are shown as red lines.

C: Location of optic chiasm and SCN (red dot) within the whole brain. Blue circle markes the hypothalamus (modified from Sobotta, 2004).

1.4.2. Circadian photoreception

For a long time, rods and cones were thought to be the receptors for circadian light input. When investigating the retinal structures necessary for the adaptation of the circadian system to light, Lucas *et al* (1999) found that loss of rods and cones had little effect on the entrainability of mice. Complete loss of the eyes, however, abolished responses to light. It was then found that a small subset (~1%) of retinal ganglion cells (RGCs) is directly (intrinsically) photosensitive and probably acts as primary photoreceptors for the circadian system. In these ganglion cells, melanopsin is expressed (Berson *et al*, 2002; Sekaran *et al*, 2003), an opsin-protein (Bellingham & Foster, 2002; Hannibal & Fahrenkrug, 2002) first discovered in melanophores of *Xenopus laevis* (Provencio *et al*, 1998) and subsequently identified in the human inner retina (Provencio *et al*, 2000). Mice lacking melanopsin and rods and cones show no significant pupil reflex and fail to entrain to light/dark cycles (Hatter *et al*, 2003; Panda *et al*, 2003). Photosensitive RGCs constantly express Melanopsin (Hatter *et al*, 2002) and the expression of melanopsin in mammalian cell lines enables photosensitivity (Melyan *et al*, 2005; Qiu *et al*, 2005). The photoresponse of RGCs in macaques, primates with a visual system similar to humans, is like that of rodents (Dacey *et al*, 2005) and a major role of the RGCs could be the detection of twighlight (Foster 2005), the transition between day and night with different wavelengths than daylight (Roenneberg & Foster, 1997) and thus a potential entraining signal. Photoresponsive RGCs directly innervate the SCN via the retinohypothalamic tract (RHT, Berson *et al*, 2002, red lines in **Fig.1.7.**) as well as other brain areas like the intergeniculate leaflet (IGL) or the olivary pretectal nucleus (OPN) which links RGCs to the pupillary light reflex (Berson, 2003).

1.4.3. The suprachiasmatic nucleus (SCN)

1.4.3.1. Determination of the SCN as the central circadian pacemaker

It took almost 20 years since the discovery of the SCN as the site of the endogenous circadian clock until its function as central pacemaker could be clearly proven. In 1988, Ralph & Menaker noted, more or less serendipitously, a Syrian hamster with a very short free running period (tau). Animals heterozygous for the tau mutation have free running periods of 22 hours, homozygous animals have a tau of 20 hours. Ralph *et al* (1990) showed that transplantation of the SCN between hamster of these genotypes led to free running periods of the donor SCN and not to that of the host. This showed that the genotype of genes expressed in the SCN determines circadian properties of a mammalian organism.

1.4.3.2. Synchronization within the SCN

As already mentioned, the SCN consists of about 20,000 neurons. Welsh *et al* (1995) showed that dissociated SCN neurons keep on firing in vitro for even weeks. Thus, each SCN neuron contains a functional clock. However, to function as a whole, a synchronization (coupling) among SCN neurons is required. There are several possible mechanisms for synchronization. One candidate transmitter is GABA (γ-aminobutyric acid) which is sufficient to synchronize SCN neurons (Liu & Reppert 2000). Also, vasoactive intestinal peptide (VIP) has been shown to be needed for circadian rhythmicity (Cutler *et al*, 2003; Colwell *et al*, 2003).

The SCN can be divided into two regions, a core and a shell, which have different properties (Lee *et al*, 2003; Hastings & Herzog, 2004). Cells of the shell show intrinsic rhythmicity but are only little innervated from the retina via the RHT (Moore *et al*, 2002). In the rat, e.g., cells in the core are directly innervated and receive light information via the RHT (Tanaka *et al*, 1997) but lack rhythmic expression of clock genes (see chapter 1.4.5.). When the majority of core cells is ablated, circadian rhythmicity is lost. SCN core cells seem to gate photic input depending on their internal phase, thus leading to differential phase shifts of rhythmic cells of the SCN shell (Antle & Silver, 2005).

1.4.3.3. Input signals of the SCN

The different responsiveness of the SCN is enabled by a Daytime and a Nighttime domain (Gillette & Mitchell, 2002) at which only certain substrates efficiently adjust clock phase (Gillette & Mitchell, 2002). In the early night, sensitivity to light is mediated by glutamate and elevation of intracellular Ca^{2+} (Honma & Honma, 2003), correlating with the timing of phase delays (Ding *et al*, 1994; Ding *et al*, 1998). In the late night, glutamate induces phase advances by a cGMP mediated pathway (Weber *et al*, 1995; Ding *et al*, 1998). During the day, responsivness of the SCN to pituitary adenylyl cyclase-activating peptide (PACAP) might modulate nocturnal responses both to light and to glutamate (Hannibal *et al*, 1997). During dawn and dusk, the SCN shows responsiveness to melatonin (Liu *et al*, 1997), a hormon produced during night in the pineal gland (Macchi & Bruce, 2004) and also in the retina (Tosini & Menaker, 1996).

1.4.3.4. Output signals of the SCN

Many physiological parameters exhibit circadian rhythmicity, e.g. body temperature, K^+ and Ca^{2+} excretion, cortisol,… but also performance depends on time of day (Aschoff 1965; Aschoff & Wever, 1981). The SCN, as the master clock, communicates with numerous brain regions to control for example the release of many hormons (e.g. corticotropin-releasing-hormone (CRH), adrenocorticotropic hormone (ACTH), Melatonin,…). Circadian rhythmicity, transmitted by the paraventricular nucleus of the hypothalamus (PVN), can reach peripheral organs (liver, gonads, fat tissue,…, Buijs & Kalsbeek, 2001). The SCN itself can also release molecules that e.g. are hypothesized to transmit behavioural circadian rhythm (Kalsbeek & Buijs, 2002; Kramer *et al*, 2001; Cheng *et al*, 2002; Gachon *et al*, 2004).

1.4.4. Peripheral oscillators

Although the SCN is the master circadian clock, most cells of the body (organs, different tissues) contain a functional circadian clock (see chapter 1.6.4.2., Hastings *et al*, 2003; Gachon *et al*, 2004; Reppert & Weaver, 2002). These clocks are usually entrained by output signals of the SCN and loss of the SCN leads to desynchonisation among peripheral clocks. Circadian rhythmicity independent of a master clock has also been found in other mammalian tissue (Bünning, 1958; Balsalobre *et al*, 2000a; Balsalobre *et al*, 2000b; Yoo *et al*, 2004; Izumo *et al*, 2003; Abe *et al*, 2002) and in insects (Hege *et al*, 1997; Plautz *et al*, 1997; Giebultowicz, 2001). Peripheral oscillators can be reset by external stimulation mimicking humoral signals (Balsalobre *et al*, 2000a; Balsalobre *et al*, 1998). *In vivo*, peripheral rhythmicity can uncouple from the SCN in restricted food protocols (Stokkan *et al*, 2001; Damiola *et al*, 2000). Peripheral clocks (e.g. liver) seem to be synchronized by the rest-activity cycle, which limits feeding time. The rest-activity cycle itself is regulated by the SCN (Cheng *et al*, 2002; Gachon *et al*, 2004). However, there are also direct ways by which the SCN can synchronize peripheral clocks, e.g. by inducing the release of glucocorticoids (Le Minh *et al*, 2001).

It is hypothesized that, besides the light entrainable circadian clock, another clock exists which can be entrained by food, the food entrainable oscillator (FEO, Stephan, 2002, see also **Fig. 1.8.**).

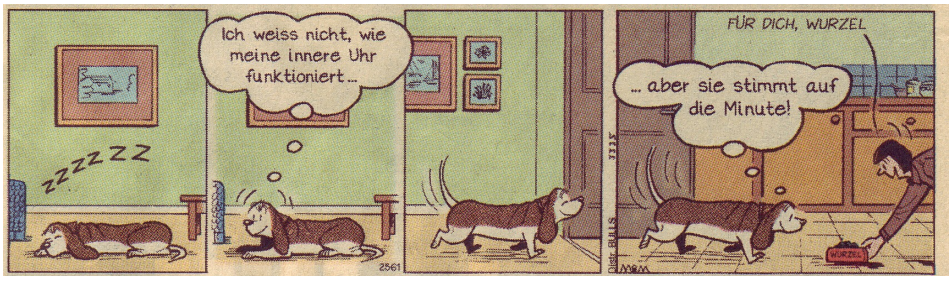


Fig.1.8.: A non-scientific report about a food entrainable oscillator (FEO). Most domestic dogs seem to be under strict control of such kind of circadian clock. (Wurzel by Alex Graham)

1.4.5. Clock genes

1.4.5.1. Clock genes from unicells to mammals

The first clock mutant has been identified in 1972 by Konopka & Benzer in *Drosophila melanogaster*, one of the most prominent organisms in genetics. The respective gene, periodical (per), was localized and cloned in 1984 by Bargiello *et al* and Reddy *et al*. Compared to unicells (e.g. Gonyaulax, Cyanobacteria), *Drosophila* is a very complex organism with many kinds of organs and tissues. In contrast to mammals (e.g. mice), *Drosophila* has a very short lifecycle (about 1 week), many descendants and thousands of flies can be kept within some square metres. Thus, it's the ideal organism for behavioural studies and many genes of the clock have been discovered by genetic screening of circadian mutants. Homologues of the *Drosophila* clock genes have been subsequently discovered in mammalian genomes, except for Clock, which has been identified and cloned first in the mouse (King *et al*, 1997).

Because this chapter is not intended to be a textbook of molecular circadian clocks and many excellent reviews have been published, only the general principle of the molecular clock will be described with an emphasis on humans. Reviews of the molecular clock and references for original publications are available for cyanobacteria (Golden & Canales, 2003; Golden, 2003), *Neurospora* (Dunlap & Loros, 2004), *Drosophila* (Hardin, 2005), and non-human mammals (Okamura *et al*, 2002; Lowrey & Takahashi, 2004; Albrecht & Eichele 2003; Rutter *et al*, 2002; Gachon *et al*, 2004; Reppert & Weaver, 2002).

1.4.5.2. The mammalian molecular circadian clock

The big question in molecular circadian research was (and still is): how can a circa 24 hour rhythmicity be maintained on the level of molecules. The basis of todays model of the circadian core clock was described by Hardin *et al* (1990) in *Drosophila*. The authors showed that PER protein feeds back on the transcription of its own RNA forming a negative feedback loop. This so called transcriptional-translational feedback loop (**Fig.1.9.A**) has been found in all other circadian model systems. The recent model of the mammalian core clock in the SCN comprises 7 components involved in various interacting feedback loops (**Fig.1.9.B and Table 1.1.**).

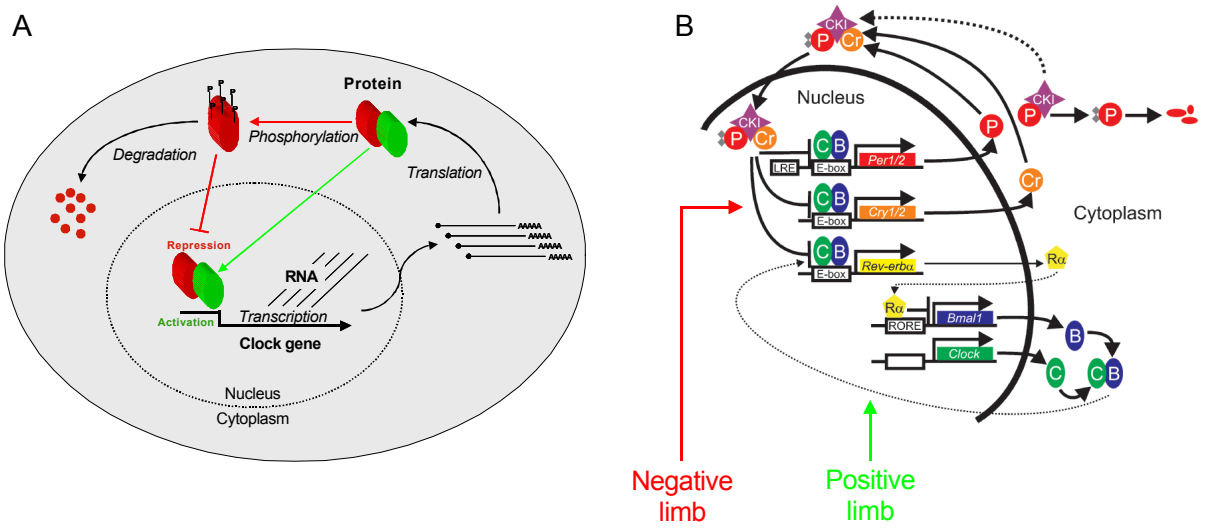


Fig.1.9.:
A) General model of transcriptional-translational feedback loops. Clock genes are transcribed and the RNA is translated in the cytoplasm. Proteins can then enter the nucleus an either activate (green) or repress (red) their own transcription or the transcription of other genes. Before entering the nucleus, some proteins are also phosphorylated, leading either to an activation or degradation.
B) Model of the mammalian molecular core clock (from Lowrey & Takahashi 2004). Genes and proteins of the positive limb are shown in green and blue (Clock and Bmal1), those of the negative limb are shown in orange and red (Cryptochrome and Period). Rev-Erbα (yellow) functions as link between both limbs. The bold black line indicates the membrane of the nucleus. For a list of genes involved in the core molecular clock see **Table 1.1.** For further information see text.
P: Period1/2; Cr: Cryptochrome 1/2; CKI: Casein kinase ε; C: Clock; B: Bmal1; Rα: Rev-Erbα; LRE: Light-responsive elements; E-Box: palindromic promotor region; RORE: Retinoic acid-related orphan receptor response element. Grey diamonds indicate phosphorylation, red elipsoids represent degraded PER.

Gene	Chromosome	Classification	Function
Clock	5	bHLH-PAS	Transcription factor
Bmal1 (Mop3, Arntl)	11	bHLH-PAS	Transcription factor
Per1	17	PAS domain	PER/CRY interaction ; Clock:BMAL1 inhibitor
Per2	2	PAS domain	PER/CRY interaction ; Clock:BMAL1 inhibitor
Per3	1	PAS domain	PER/CRY interaction
Cry1	12	Flavoprotein	Interaction with PERs ; inhibition of CLOCK:BMAL1
Cry2	11	Flavoprotein	Interaction with PERs ; inhibition of CLOCK:BMAL1
Rev-Erbα	17	Orphan nuclear factor	Inhibitor of Bmal1 ; links negative and positive feedback loops
CKIε	22	Casein kinase	Phosphorylation of PERs, CRYs and BMAL1

Table 1.1.: Overview of the mammalian molecular core clock components. The locations on human chromosomes and the classification of protein families are shown. Per: Period; Cry: Cryptochrome; CKIε: Casein Kinase Iε; BHLH: Basic helix-loop-helix; PAS: Period-Arnt-Single-minded (from Lowrey & Takahashi 2004)

The molecular mechanism shown in **Fig.1.9.** is held by all SCN neurons and functions autonomously as long energy is provided (see 1.4.3.2.) but the molecular clocks can be entrained to the ambient light-dark cycle. After light from the retina has been transmitted via the RHT to the SCN (Reppert & Weaver 2002), intracellular signalling pathways enhance the transcription of Per RNA (via binding of the LRE, **Fig.1.9.B**, Honma & Honma 2003). The general activator of Per and Cry is the heterodimer BMAL1:CLOCK which activates the transcription of Per, Cry, and Rev-Erbα (**Fig.1.9.B**, positive limb). Per and Cry RNA is translated in the cytoplasm and accumulates, forming PER:CRY heterodimers. Simultaneously, PER is phosphorylated by Caseine Kinase Iε (CKIε, **Table 1.1.**, Lowrey *et al*, 2000), leading partly to

a degradation of PER. The heterodimer of PER:CRY can enter the nucleus and repress the activation of its own transcription (**Fig.1.9.B**, negative limb). After entering the nucleus, REV-ERBα represses the transcription of Bmal1 by binding the RORE element in the promotor region of Bmal1. Thus, BMAL1 attenuates its own transcription by activation of Rev-Erbα. As an exception, Clock is constitutively expressed and unaffected by rhythmicity of the other clock components.

In summary: I) CLOCK:BMAL1 activates Per, Cry, and Rev-Erbα II) PER:CRY inhibits its own transcription and the transcription of Rev-Erbα and REV-ERBα inhibits the transcription of Bmal1 III) Rev-Erbα is inhibited by PER:CRY leading to a cessation of Bmal1 inhibition by REV-ERBα IV) inhibition of Per/Cry transcription leads to a decrease of PER:CRY production and the inhibition of Per/Cry transcription. Da capo ad libitum! Once understood, this is not as complicated as it looks on first sight.

Another negative feedback loop interlocking with the Per loop (not shown in **Fig.1.9.B**) is formed by Dec1 and Dec2 (Honma *et al*, 2002; Honma & Honma, 2003). Several clock controlled genes (CCGs) are rhythmically transcribed, activated by cycling products of the molecular clock. Although the general mechanism of the molecular clock is assigned to transcriptional-transational feedback loops, a Japanese group recently found a persisting 24 hour rhythm in the cyanobacterium *Synechococcus elongatus* without cycling RNA of the respective genes (Tomita *et al*, 2005; Roenneberg & Merrow 2005a).

For a more detailed description of the mammalian core molecular clock, mutations of clock genes and genetic approaches see Lowrey & Takahashi (2004). Rutter *et al* (2002) integrate entrainment, clock molecules, and metabolism, showing differences and similarities of molecular clocks outside the SCN. Similarities and differences between organisms are presented in a very recent comparative review by Bell-Pedersen *et al* (2005).

1.4.6. A net of oscillators

1.4.6.1. Different levels of oscillations

As already mentioned in chapter 1.4.3.2., cells of the SCN can synchronize to each other. Rhythmic outputs of the SCN synchronize, e.g., melatonin secretion from the pineal gland which feeds back on the SCN (**Fig.1.10.A**, Gillette & Mitchell, 2002). Also, via control of activity, feeding is restricted to certain times. Availability of food can also synchronize peripheral clocks (**Fig.1.10.A**, Roenneberg & Merrow, 2005c; Gachon *et al*, 2004). Cyclically released hormons, controlled by the SCN, can directly entrain peripheral oscillators of different organs (**Fig.1.10.A**).

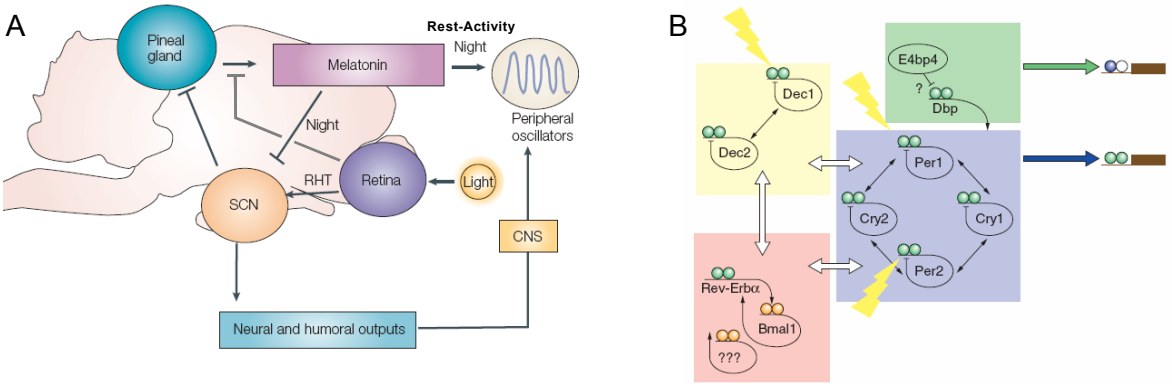


Fig.1.10:
A) Coupling of different areas of the brain and synchronization of peripheral oscillators (modified from Bell-Pedersen *et al*, 2005).
B) A hypothetical model of the molecular core clock components shown in **Fig.1.9b** represented as distinct, interacting feedback loops. Flashes indicate clock components directly inducable by light inputs from the RHT. Rhythmic induction of output genes is represented by green and blue arrow. Green speres: CLOCK:BMAL heterodimer; yellow spheres: unknown transcription factor; Dbp: D-box binding protein (from Roenneberg & Merrow, 2003).

While whole anatomical parts can form feedback loops (e.g. SCN and pineal gland), this could also be the case on the molecular level (**Fig.1.10.B**, Roenneberg & Merrow, 2003). Computational models show that several interlocked feedback loops can maintain a circa 24 hour rhythmicity (Roenneberg & Merrow, 2002; Leloup & Goldbeter, 2003; Geier *et al*, 2005). Single feedback loops can have ultradian cycle lengths and dampen without further stimulation. The overall rhythmicity depends on several parameters like production and degradation rate and coupling strength (Roenneberg & Merrow, 2002).

1.4.6.2. Morning-Evening (M-E) oscillator

In 1976, Pittendrigh & Daan (1976b) hypothesized two distinct oscillators that differently respond to light, depending on time of the day. One is responsive to morning light, one to evening light. This kind of pacemaker could accommodate to seasonal changes by directly measuring day (or night) length. Molecular and neurohistochemical results give rise to further speculations on that issue. Daan *et al* (2001) suggested that known components of the molecular clock (Per1 & Cry1 and Per2 & Cry2) could form two separate oscillators, one locking to dawn (Per1/Cry1), one locking to dusk (Per2/Cry2). Jagota *et al* (2000) found morning and evening oscillations of Per in different mouse SCN slices. In *Drosophila*, different groups of neurons control the morning and evening activity bouts. Ablation of one or the other group of neurons suppressed either the morning or evening anticipatory activity peak (Stoleru *et al*, 2004; Grima *et al*, 2004). Although these results strengthen the M-E hypothesis, substrates for E or M oscillators still have to be found.

1.5. Human chronobiology

1.5.1. Pros and Cons of human circadian research

In (chrono) science, there are advantages and disadvantages for every organism under investigation. *Drosophila* is an ideal organism in terms of a life cycle that allows extensive crossing experiments and a body size small enough to be kept in small vessels (Brookes, 2002). On the other hand, there is no possibility for long term experiments and the measurement of physiological parameters is extremely difficult. The reverse is true for humans. Additionally, human circadian research is very expensive and labour-intensive. First, one has to find appropriate candidates. Second, appropriate people have to be willing to deal with experimental conditions. But humans can give detailed information and field studies under natural conditions are very feasable with humans. The mouse combines the advantages of flies and humans (exept for detailed information of course) and is thus one of the most commonly used organisms in circadian research.

1.5.2. Endogenous human circadian rhythms

By 1960, basic circadian principles were known for most organisms from unicells, insects, birds, and mammals (Aschoff, 1960; Pittendrigh, 1960). Almost nothing was known about humans. An endogenous rhythm of body temperature has been found in monkeys (Simpson

& Galbraith, 1906,). Some other physiological parameters have been shown to exhibit diurnal rhythms (Pincus, 1943; Aschoff, 1955), however, the source of rhythmicity remained unknown. In 1962, Aschoff & Wever first showed that humans maintain rhythmicity of rest and activity, rectal temperature, and urine constituents when isolated from social, temporal, and light influences. However, the measured period of the rhythmicity was not exactly 24 hours but about 25 hours. By this time, any potential influence on circadian rhythmicity had to considered, therefore the experiment was performed in a second world war bomb shelter in Munich. From 1965 on, the new 'bunker' in Erling, Andechs was used. Many isolation studies were also performed in natural caves and in other facilites around the world (for overview see Wever, 1979).

These experiments showed that behavior (rest-activity, sleep-wake) and physiology (body temperature, potassium excretion, etc.) exhibit an endogenous rhythmicity. Additionaly, rhythms can uncouple and cycle with different periods. In some subjects investigated, this happened spontaneously in constant conditions. The temperature continued to cycle with about 25 hours while rest-activity rhythms differed greatly (between 18 and 34 hours. The phenomenon is called 'Internal desynchronization' (Aschoff, 1965; Aschoff *et al*, 1967; Wever, 1979; **Fig.1.11.A**).

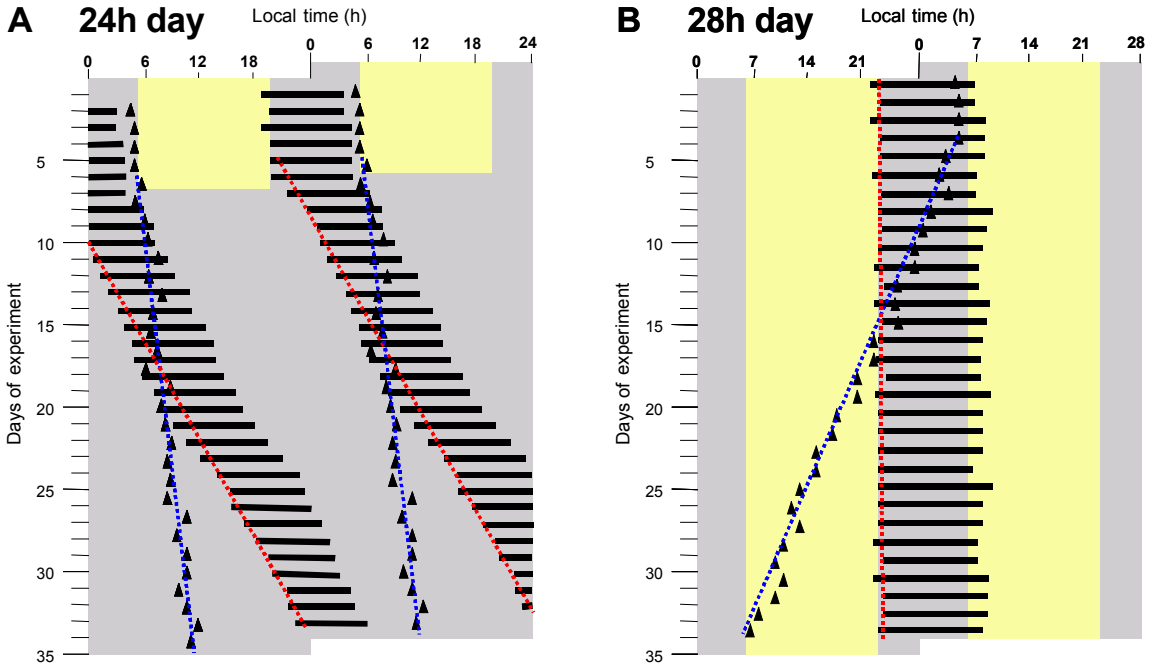


Fig.1.11.: A hypothetical example of internal desynchronization (left figure) and partial entrainment (right figure). Yellow area indicates light, grey area indicates darkness.
A) Internal desynchronisation: In some cases of free running circadian rhythms in humans it happened that the sleep-wake cycle lengthened (red dotted line) while the rhythm of rectal body temperature continued to run free with a period of about 24 hours (blue dotted line). In human free running experiments, participants of course are not released in constant darkness but in an environment without any information about outside life. Therefore, grey area from day 7 on does not indicate constant darkness but constant environmental conditions.
B) Partial entrainment: When applying an artificial day with e.g. 28 hours (right figure), the body temperature rhythm cannot adapt to such an extreme long period (blue dotted line) and uncouples from the sleep-wake cycle which can adapt the 28 hour day (red dotted line). The application of either very long or very short T-cycles is used in the forced desynchrony protocol (see text). **Note that the artificial day in B) contains 28 hours but the ratio of light and darkness is the same as in the first 6 days of A).**

The sleep-wake rhythms can adapt to very long or short T-cycles (20 or 28 hours). These extreme periods are outside the range of entrainment of body temperature or plasma melatonin and the rhythms uncouple (**Fig.1.11.B**). The 'Forced desynchrony protocol' was introduced to face internal and external effects on the circadian pacemaker (for references see Czeisler *et al*, 1999). Free running experiments showed human internal rhythmicity to have a tau of about 25 hours (Aschoff & Wever, 1962; Wever, 1979). By using the 'Forced desynchrony protocol', Czeisler *et al* (1999) showed that the intrinsic period of the human circadian pacemaker is on average 24.18 hours which very close to 24 hours.

1.5.3. Entrainment of human circadian rhythms

In the beginning of human circadian research, non-photic social cues were thought to be most important for the entrainment of humans. Non-photic entrainment such as scheduled sleep-wake cycle, exercise, mealtimes, or social contacts has been shown to have some effect on human circadian rhythms. (Mistlberger & Skene, 2004; Mistlberger & Skene 2005). However, compounding effects of light always have to be considered. For example, one publication reported a phase shift after illumination of the back of the knee (Campbell & Murphy, 1998). As in other mammals, light exclusively reaches the circadian pacemaker via the retina (see chapter 1.4.1.) and a subsequent study refuted these results showing that even very dim light is capable of entraining circadian rhythms which has been the entraining effect and not light behind the knees (Wright & Czeisler, 2002). Because the compounding effects of light are very difficult to control, totally blind people are ideal subjects to study (Mistlberger & Skene 2005).

Today, light is accepted to be the most important zeitgeber for the entrainment of mammalian circadian rhythms (Duffy & Wright, 2005). The mechanism how light entrains the human clock, however, seems to be very complex. Phase response curves (PRCs, see 1.3.2.2.) are available also for humans and there is no evidence for a 'dead zone' in which the clock is not responsive to light (Jewett *et al*, 1997). Besides the time of light exposure, intensity, prior light history, and spectral composition might play an important role in entrainment (Duffy & Wright 2005).

Short wavelength (blue) light most effectively suppresses melatonin (Thapan *et al*, 2001) and is more effective in producing phase shifts than long wavelength light (Warman *et al*, 2003; Lockley *et al*, 2003). Twilight consits mostly of short wavelength light, thus, the transition of day and night could play an important role in entrainment (Roenneberg & Foster, 1997).

The human circadian system can integrate short episodes of light exposure and e.g. can be advanced by several short light pulses (Kronauer *et al*,1999,) and brief light pulses can en-

train as effectively as continuous light exposure (Rimmer *et al*, 2000; Gronfier *et al*, 2004). Entrainment could also depend on prior light history. Smith *et al* (2004) showed that melatonin suppression depends on recent photic history and the photic circadian system could be adaptive to light history (Fain *et al*, 2001).

Recent research revealed many new insights into the human (mammalian) circadian system. However, many questions are still open, especially how the clock is entrained in the real world, outside laboratory facilities, is far from being understood (Duffy & Wright, 2005).

1.5.4. Investigating human endogenous rhythms

Many physiological and biochemical parameters such as body temperature, melatonin, cortisol, heart rate, blood pressure,… exhibit circadian rhythms (Aschoff & Wever, 1981; for overview see Lemmer, 2004). However, endogenous circadian rhythms in humans are masked by numerous environmental factors. In order to dissect endogenous and exogenous components of a circadian rhythm, the constant routine protocol has been invented (described by Waterhouse & DeCoursey, 2003). In a constant routine, exogenous components are minimized by strict conditions. Subjects have to stay awake lying down for 24 – 40 hours under constant temperature, humidity, and dim light conditions. Isocaloric meals are taken at regular intervals (Duffy & Dijk, 2002). Core body temperature, e.g., is still rhythmic in a constant routine but with a smaller amplitude, indicating a strong exogenous component. Blood pressure is rhythmic only under natural conditions, thus almost completely of exogenous cause (Waterhouse & DeCoursey, 2003). The constant routine protocol has been adjusted to special needs in many studies and is a common tool for the investigation of human circadian rhythms (Duffy & Dijk, 2002).

1.5.5. Sleep and (circadian) sleep disorders

1.5.5.1. Sleep basics

Sleep is commonly regarded as the opposite of wakefulness. Actually, many different areas of the brain are very active during sleep (Pace-Schott & Hobson, 2002; Hobson & Pace-Schott, 2002). Sleep can be roughly divided into two different types: REM (Rapid Eye Movement) and Non-REM sleep, also called slow-wave-sleep (SWS) and which is further divided into stages I – IV. Different types of sleep are defined by characteristic polysomnographic measurements (see Pace-Schott & Hobson, 2002, Box1).

Sleep is associated with many cognitive functions and capabilities (Hobson & Pace-Schott, 2002) and implicated with brain plasticity, learning and memory (Maquet, 2001), or discrimi-

nation skills (Gais *et al*, 2000). Other functions of sleep are also hypothesized, e.g. energy conservation, restoration and detoxification of the brain (Maquet, 2001).

1.5.5.2. The two-oscillator model of sleep and wakefulness

Besides the circadian component of (the timing of) sleep, there is the 'restorative sleep theory' (Pinel, 2001). The circadian theory assesses sleep as an energy saver when the organism has to be inactive anyway. The restorative theory assumes a restoration of a physiological homeostasis (Daan & Beersma, 1984). Actually, both theories seem to be important and Borbély (1982) suggested "A two process model of sleep regulation" (see also Daan *et al*, 1984). The model in **Fig.1.12.** shows how two hypothetical oscillators, a circadian (CO) and a homeostatic one (HO, also called 'Hourglass oscillator'), might interact to establish a sleep-wake cycle. During the day, the propensity to fall asleep is controlled by the HO. This is counteracted by the increasing wake propensity, controlled by the CO (Dijk & Czeisler, 1994). At the end of the biological day, before the onset of melatonin, the CO controlled wake propensity decreases,opening a gate to sleep (for melatonin functions in humans see Claustrat *et al*, 2005). After sleep onset, the propensity for sleep, controlled by the HO, decreases and is counteracted by the drive for sleep, controlled by the CO, which reaches its maximum around the body temperature nadir (Dijk & Czeisler, 1994). Towards the end of sleep, REM sleep density increases, controlled by the CO and the HO. A high density of REM sleep then promotes waking about two hours after the body temperature nadir (Åkerstedt *et al*, 2002; Dijk & von Schantz, 2005). Different neurotransmitters seem to be involved in these regulatory mechanisms. Prolonged wakefulness induces high concentration of adenosine which could promote sleep by negatively feeding back on neuronal activity (Porkka-Heiskanen *et al*, 2002). Orexin, secreted from cells of the lateral hypothalamus, increases wakefulness in a dose dependent manner (Pace-Schott & Hobson, 2002) and might play a role in human narcolepsy (Taheri & Mignot, 2002). Another neurotransmitter that modulates circadian rhythms and sleep is serotonin, secreted by serotonergic cells in the raphe nuclei of the brain stem (Ursin, 2002).

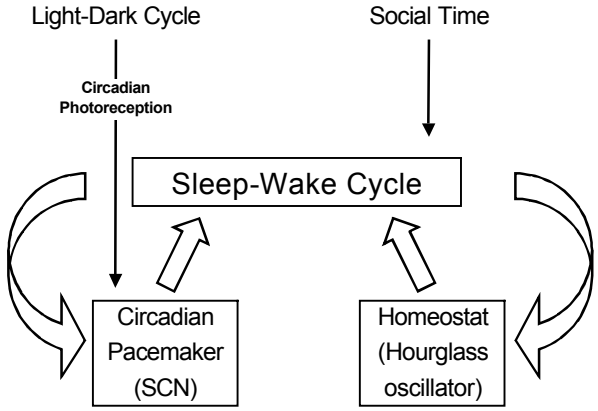


Fig.1.12.: Simplified model of the interplay of circadian pacemaker, sleep homeostat (for details see text), daily light-dark cycle, and social influences in the regulation of the sleep-wake cycle (redrawn from Dijk & von Schantz, 2005). Social time represents influences affecting only the sleep-wake cycle.

The gating of sleep thus depends on the phase relationship of both oscillators (Daan *et al*, 1984). When the sleep wake cycle is willingly shifted ('Social Time' in **Fig.1.12.**), light input is altered ('Light-Dark-Cycle' in **Fig.1.12.**) and this feeds back on the CO. In the rat, the activity of the SCN is modulated by different states of sleep (Deboer, 2003) forming a direct feed-back from the sleep-wake cycle on the CO.

While many genes underlying the CO have been discovered in humans (see chapter 1.5.5.), only few is known about the genetic background of the HO (Naylor *et al*, 2000; Franken *et al*, 2001).

1.5.5.3. Circadian rhythm sleep disorders (CRSD)

Sleep seems to be defined by two major parameters: its timing within the 24 hour day and its duration. Numerous sleep disorders have been described (Hohagen, 1999; Taheri & Mignot, 2002; International classification of sleep disorders, 1990). A subtype of sleep disorders, the circadian rhythm sleep disorders (CRSD) does not affect directly sleep itself but rather the timing of sleep. Dagan (2002) discriminates six subgroups of CRSD: Advanced sleep phase syndrome (ASPS), Delayed sleep phase syndrom (DSPS), Non-24-h-Wake Syndrome (Non-24), Irregular sleep pattern, Shift work, and Jet lag. The latter two are of exogenous cause and thus a social and not a biological problem. Non-24 and irregular sleep pattern are most common in blind people (Cermakian & Boivin, 2003).

ASPS and DSPS caught major interest of circadian and sleep research. People suffering from DSPS fail to fall asleep at conventional sleep times but when not dependent on any schedule, have a refreshing sleep with spontaneous awaken (Weitzman *et al*, 1981). ASPS 'patients' fall asleep in the early evening with spontaneous awakening in the early morning (Jones *et al*, 1999). In both cases, affected people do not complain about sleep disturbancies when not restricted to a conventional sleep-wake schedule (Dagan, 2002). Based on a 9 to 5 working day, conventional sleep times could be 23:00 to 7:00. DSPS patients usually sleep from 4:00 ± 1h to 12:00 ± 1h without any restrictions. This obviously collides with a 9 to 5 working schedule, thus, these people in most cases suffer from restricted sleep due to late sleep onset and forced rise times in the morning. ASPS people fall asleep between 19:00 and 20:00 and spontaneously awake between 3:00 and 4:00. Because social life often takes place in the evening, the spontaneous schedules of these people does not collide with work times but rather with the social environment.

There have been many attempts to treat DSPS in order to establish conventional sleep times. Czeisler *et al* (1981) imposed a 27 hour day with strict schedules on affected people to shift them 'through the day' by delaying sleep times. In some cases, a stable sleep-wake rhythm from about 0:00 to 7:00 could be maintained for several months. Treatments with Vi-

tamin B_{12} (Okawa *et al*, 1990), melatonin (Dahlitz *et al*, 1991), or light therapy (Rosenthal *et al*, 1990; Lewy & Sack, 1986) also could, to a certain extent, improve DSPS towards conventional sleep times. Nevertheless, people affected by DSPS more often show psychological problems like depression and other emotional features (Shirayama *et al*, 2003). The question is, however, if these psychological symptoms are linked to the circadian clock or to the difficulties in coping with common schedules. There is only little literature on ASPS (e.g. Billiard *et al*, 1993), maybe due to the fact that people affected by ASPS rarely seek help in sleep clinics.

One cause of ASPS and DSPS could be circadian pacemakers with free-running periods that deviate largely from 24 hours (shorter in ASPS, longer in DSPS) and thus entrain to the 24 hour light-dark cycle with extreme sleep times (**Fig.1.6.**, Jones *et al*,1999; Weitzman *et al*, 1981).

1.5.6. Human clock genes

For all clock genes presented in **Table 1.1.**, human homologs have been discovered. This started the run on the identification of specific allels underlying human chronotypes. **Table 1.2.** lists polymorphisms in human clock genes identified in people with sleep disorders and / or diurnal preference.

	Mutation	Phenotype	Age	n	Questionnaire	Sleep log, etc
Katzenberg et al 1998	**hClock:** 3111	Late	50.0 ± 7.0	410	H-Ö	--
Katzenberg et al 1999	**hPer1:** A to G (2548)	--	46.0 ± 0.4	463	H-Ö	--
Pedrazzoli et al 2000	**hTimeless:** Q831R	--	50.3 ± 7.8	528	H-Ö	--
Toh et al 2001	**hPer2:** S662G	ASPS *	20 - 69	23 (Family)	H-Ö *	3 weeks (+actigraphy) *
Ebisawa et al 2001	**hPer3**: H4 Haplotype	DSPS **	28.4 ± 10.0	48	**	**
Robilliard et al 2002	**hClock:** 3111	--	35.0 ± 13.0	484	H-Ö	1 Night
Archer et al 2003	**hPer3:** Length polymorphism	Late / DSPS	35.0 ± 13.0	484	H-Ö	1 Night
Xu et al 2005	**CKIδ:** T44A	ASPS *	20 - 65	5 (Family)	*	*
Carpen et al 2005	**hPer2:** SNP in 5'UTR	Extreme	35.0 ± 13.0	484	H-Ö	1 Night

Table 1.2.: Overview of mutations identified in genes directly associated with the human molecular core clock. Timeless is an essential part of the *Drosophila* core clock and a homolog has been found in the human genome. However, a role in the human core clock could not be shown (Bell-Pedersen *et al*, 2005). For exact classification conditions see Jones *et al* 1999 (*) and the International classification of sleep disorders 1990 (**).

Although most of these studies show associations between polymorphisms (Single Nucleotide Polymorphism, SNP or length polymorphism) and circadian phenotypes, not all mutations can be assigned to all subjects within the same phenotype. The link between the 3111 Clock polymorphism detected by Katzenberg *et al* (1998) were not found in other populations (Robilliard *et al*, 2002; Iwase *et al*, 2002). The mutation in the hPer2 phosphorylation site (see chapter 1.4.5.2.) linked to familiar ASPS (Toh *et al*, 2001) was not detected in all affected family members. This indicates that besides the polymorphisms detected, other

polymorphisms (SNPs) or genetic factors could be involved (Piggins, 2002). On the other hand, the length polymorphism in hPer3 detected in a british sample by Archer *et al* (2003) has recently been confirmed in a brazilian sample by Pereira *et al* (2005). The authors also suggested a role of latitude in the influence of the polymorphism on DSPS.

Screens in human clock genes have also been performed in patients with bipolar disorder (hPer2, Shiino *et al*, 2003) and major depression (hClock, Desan *et al*, 2000). The hPer1 gene has been extensively studied in terms of structure (Taruscio *et al*, 2000), transcriptional regulation (Motzkus *et al*, 2000; Motzkus *et al* 2002), and phosphorylation by hCKIδ (Camacho *et al*, 2001) and CKIε (Keesler *et al*, 2000; Vielhaber *et al*, 2000). Another period gene in humans (hPer4) has been shown to be a pseudogene (Gotter & Reppert, 2001).

Considering that people suffering from ASPS and DSPS usually do not show sleep disturbencies (in case of no restricting schedules), one could ask if ASPS and DSPS should be seen as a disorder or rather as the extreme ends of a (almost) normal distribution of chronotype (as shown by Roenneberg *et al*, 2003a). A normal distribution of a trait usually reflects an interplay of many genes and respective alleles (Strachan & Read, 2004). This and the contrary results on human clock gene mutations mentioned above could imply that not a single polymorphism makes up a phenotype (like e.g. in sickle-cell anemia) but a certain combination of polymorphisms in many genes. Also, polymorphisms could be population dependent and the same chronotype in different populations could be due to a different constellation of polymorphisms.

1.5.7. Chronobiology and health

1.5.7.1. The circadian clock in modern times

The circadian clock in the SCN is the master coordinator (pacemaker) of other body clocks (Reppert & Weaver, 2002; Rutter *et al*, 2002). Usually, behaviour depends on time of the day or rather to the environmental light-dark cycle, e.g. activity starts in the morning, food as breakfast, lunch, and dinner, rest in the evening, sleep at night, etc. The circadian master clock of the SCN recognizes the light-dark cycle via the eyes and receives feedback from the organism, e.g. via melatonin, excreted by the pineal gland (**Fig.1.10a**). The resulting temporal programme in a highly coordinated interplay of behaviour, physiology, and biochemistry is embedded in the 24 hour light dark cycle (Hastings *et al*, 2003; Reppert & Weaver, 2002; Rutter *et al*, 2002; Gachon *et al*, 2004).

The biological clock of humans and their ancestors was mainly influenced only by the daily change of light and darkness for millions of years. Only with the invention of artificial light, and consequently with humans working around the clock, a 24 hour availability of everything,

and flights around the world, the clock was exposed to rapidly changing lighting conditions. Although the circadian clock can adjust to these changes, an on-time-shift of several hours, be it due to a transatlantic flight, or to changing shift work schedules, can not be compensated within short time. The clock adapts to the new light regime with special characteristics (see chapter 1.3.2., Roenneberg *et al*, 2003b). The speed of adaptation depends on strength and timing of the zeitgeber and is approximately one day per shifted hour. This is not a big deal when travelling over time zones ones or twice a year for vacation but long-term shifting weekly or even daily by several hours can cause health problems (Foster & Wulff, 2005).

1.5.7.2. The circadian clock and pathology

Generally, the circadian clock can be linked in four ways to pathologies, be it physiological or psychological: a) Its course exhibits circadian variation, b) it is caused by a malfunction of the circadian clock, c) a disrupted interplay of circadian clocks and environmental light-dark cycle can lead to or at least promote a pathology (Klerman, 2005; Foster & Wulff, 2005) or d) internal desynchronisation.

a) Circadian rhythm of diseases

Episodes of angina pectoris (Cannon *et al*, 1997) or the risk for acute myocardial infarction (Cohen *et al*, 1997) is more pronounced in the morning when blood pressure increases. The circadian rhythmicity of cardiovascular diseases has been extensively studied (Hastings *et al*, 2003) and research in a special field of chronoscience – chronopharmacology – showed daily rhythms in numerous other syndroms like asthma, allergic reactions, pain, epilepsy (for overview and references see Lemmer, 2004). A specially designed, chronotherapeutic medication, e.g. chemotherapeutics, can improve pharmacological treatment of diseases and reduce side effects. Although highly recommended (Hassler & Burnier, 2005; Lemmer, 2000), a general chronotherapeutic medication is almost not feasable within a normal clinics daily routine.

b) From the circadian clock to diseases

Abnormalities of the circadian clock can lead to diverse sleep disorders (Wijnen *et al*, 2005). There is evidence that malfunctions of the circadian clock also cause other syndroms besides sleep disorders. In mice, a mutation in the Clock gene is linked to altered feeding be-

haviour and the 'metabolic' syndrome (Turek *et al*, 2005). A variation of Per2 is associated with increased alcohol consumption in mice and humans (Spanagel *et al*, 2005).

The circadian clock is linked to cell-cycle regulation in perpipheral tissues (Fu & Lee, 2003) and gates cytokinesis (Nagoshi *et al*, 2004). The risk for cancer is much higher in Per2 mutant mice, probably due to a deregulation of tumor suppressor genes (Fu *et al*, 2002). In humans, variations of clock genes have also been associated with increased risk for cancer. A length polymorphism in Per3 is linked with an increased risk for breast cancer in premenopausal women (Zhu *et al*, 2005). A disturbed expression of Per1, Per2, and Per3 has been found in breast cancer tissue but not in non-cancer cells (Chen *et al*, 2005). A higher risk for cancer has not only been observed in cases of circadian clock abnormalities but also in association with disrupted circadian rhythms, especially shift work (see text below).

c) Shift work and sleep dept

The term 'jet lag' is usually applied to transatlantic flights. But someone is also jet lagged after changing from normal day shift to night shift, working during the subjective night and subsequently sleeping during the subjective day. In both cases, behavioural rhythms and the natural light-dark cycle are uncoupled. Nightshift is probably the most abnormal way of working in terms of the circadian clock and results in performance deficits, decreased safety and productivity (Folkard & Tucker, 2003) and increased risk for accidents during work and on the way home after work (Foster & Wulff, 2005). Also, shift work is associated with numerous syndroms like gastrointestinal and cardiovascular disease, metabolic disturbances (Knutsson, 2003), somnolence during work and sleep deficiencies (Åkerstedt, 2003). In consequence of this, deficient sleep can have a negative impact on mental health, cognitive and immune function (Foster & Wulff, 2005).

There is evidence that cancer is also linked to disruptions of the circadian clock, as happens during shift work (Schernhammer *et al*, 2001). A possible link between disrupted circadian rhythms and cancer could be melatonin which has been shown to have antiproliferative effects on tumors (Stevens, 2005; Claustrat *et al*, 2005). An increased risk for breast cancer among nurses working night shift has been shown by a meta-analysis on publications from 1960 to 2005 (Megdal *et al*, 2005).

Not only shift work but also early work schedules collide with the circadian clock of the majority of people. Most people accumulate sleep dept during the work week for which they compensate on free days (Roenneberg *et al*, 2003a). Restricted sleep results in symptoms mentioned above. Younger people are, on average, later later types, thus suffering even more from early school or work schedules (Roenneberg *et al*, 2004; Yang *et al*, 2005). Although adolescents are on average later types, in Germany, school starts at about 8:00 in

the morning, sometimes even earlier. In many cases, this leads to sleep loss and impaired performance and could be prevented by later school schedules (Roenneberg & Merrow, 2005b; Roenneberg, 2004b).

1.6. Morningness-Eveningness vs Chronotype

1.6.1. The Horne-Östberg Morningness-Eveningness questionnaire (MEQ)

The easiest method to investigate human circadian behaviour is to simply ask how people spend their days, especially about their sleeping habits.

Up to date, the most commonly used questionnaire concerning daily preference is the Morningness-Eveningness questionnaire (MEQ) by Horne & Östberg (1976). The MEQ (and diverse derivatives, see Roenneberg *et al*, 2003a) have been used to assess the tendency towards being rather a morning type (lark) or an evening type (owl) in many studies with psychological, medical, work scientific, and sociological background (Natale *et al*, 2005; Murray *et al*, 2003; Steele *et al*, 2005; Nebel *et al*, 1996; Valdez *et al*; 1996; Taillard *et al*, 2001; Taillard *et al*, 1999; Adan & Natale, 2002; Natale & Adan, 1999; Natale *et al*, 2002).

Questions of the MEQ are mostly subjective (e.g. alertness in the morning) with four different choices. Some questions ask for given ranges of time (e.g. at what time in the evening do you feel tired and as a result in the need of sleep?). Every answer is supplied with a score, low scores indicating eveningness and high scores indicating morningness. The morningness-eveningness (M-E) scale ranges from 16 (extreme late) to 86 (extreme early). The original publication divided the score into five groups: Definitely morning type (70-86), Moderately morning type (59-69), Neither type (42-58), Moderately evening type (31-41), and Definitely evening type (16-30). The MEQ has been validated by measuring oral temperature using 150 people between 18 and 32 years ($n♀ = n♂$, Horne & Östberg, 1976).

The MEQ score correlates with actual behavioural and physiological parameters. Individuals classified as 'morning types' entrained their wake time to an earlier hour than 'late types' (Duffy *et al*, 2001). Correlations with physiological rhythmicity could be shown for melatonin (Duffy *et al*, 1999), cortisol (Bailey & Heitkemper, 2001), and body temperature (Baehr *et al*,2000; Duffy *et al*, 1999).

1.6.2. Disadvantages of the MEQ

The MEQ score seems to reflect the phase of an individuals circadian clock. In many studies, the MEQ score is compared with subjective scores of psychological questionnaires (e.g. Seasonal pattern assessment questionnaire, SPAQ, Natale *et al*, 2005; Positive and negative affect scale, PANAS, Murray *et al*, 2003). In this cases, a relative position on a subjective M-E scale is sufficient to correlate with other subjective scales (without physical units). However, melatonin onset or trough of body temperature are measured either as time points (e.g. 6:00) or as phase (e.g. 60^0). While the scale of the MEQ is based on subjective assessment, physiological parameters are physically measured.

In the 1990s, huge progress has been made in the discovery of clock genes (see chapter 1.4.5.). Although no clock gene has directly been discovered in humans, most clock genes from *Drosophil*a or mouse have homologs in the human genom and very often similar functions and mutations in human clock genes have been associated with diurnal preferences or circadian rhythm sleep disorders (chapter 1.5.4.3., Table 1.2., Dagan, 2002; Cermakian & Boivin, 2003). In most studies, the MEQ has been used to determine the tendency towards morning-, evening-, or, neither type (Katzenberg *et al*, 1999; Archer *et al*, 2003; Katzenberg *et al*, 1998; Pedrazzoli *et al*, 2000; Robilliard *et al*, 2002; Carpen *et al*, 2005).

Dijk *et al* (2000) and Roenneberg *et al* (2004b) showed that properties of the endogenous circadian clock as well as actual sleep times vary greatly throughout life. The cutoffs determined in the original publication (Horne & Östberg, 1976) are based on a sample of people between 18 and 32 years. Recently, Taillared *et al* (2004) validated the MEQ in a middle-aged population of french workers (n=566, Age: 51.2 ± 3.2 years). They actually found that only three groups can be defined from this sample and they suggested new cutoffs to determine groups (Evening type: ≤ 52, Neither type: 53 – 64, Morning type: ≥ 65). The MEQ score thus seems to be inconsistent between younger and older age groups and also between populations (Caci *et al*, 2005).

Roenneberg *et al* (2003) and Valdez *et al* (1996) reported different sleep-wake behaviour between work days and free days. On work days, the majority of people accumulate sleep dept, most probably due to early working schedules for which they compensate on free days. The MEQ does not allow to discriminate between work days and free days and individual participants could relate questions to either one or the other situation.

Summarizing, there are several disadvantages of the MEQ: first, only a qualitative assessment is possible. This is problematic in case of genetic analysis (see later in text). Second, the MEQ score does not seem to be consistent when investigating samples of different age. And finally, there is no discrimination between work day and free days.

1.6.3. Questionnaires in the context of quantitative genetic analysis

1.6.3.1. Qualitative vs quantitative scale

Many socio-medical problems of today can be related to the circadian clock (Foster & Wulff, 2005). One big problem is shift work which, depending on schedules applied, forces people to change their sleeping habits weekly by several hours. Also, severe circadian rhythm disorders like ASPS (Jones *et al*, 1999), DSPS (Weitzman *et al*, 1981) put much pressure on affected individuals when having to cope with social and/or working schedules. One aim in human circadian research is the identification of genes (and the respective phenotype-related alleles) contributing to the circadian clock with the ultimate goal to develop customized pharmacolocical treatments for jet lag and sleep disorders. However, the molecular machinery of the mammalian circadian clock seems to be far more complex then thought years ago and much more unknown genes could be involved (Shimomura *et al*, 2001; Roenneberg & Merrow, 2003). The more genes and respective alleles, the more the distribution of allelic combinations resembles a normal gaussion distribution (Strachan & Read, 2004). In case of quantitative genetics, a qualitatively assessed chronotype or an assessment of even just the tendency towards morning type or evening type might be only a rough estimate.

1.6.3.2. Human chronotype is heavily masked by environmental influences

In contrast to experiments with animals, fungi, or plants under laboratory conditions, human circadian behaviour is highly influenced by various factors and can be altered at will. It is, therefore, important to relate chronotypes to their respective environment. To face deficiencies of common chronotype assessment, the Munich ChronoType Questionnaire (MCTQ) has been invented (Roenneberg *et al*, 2003a). A chronotype dependent discrepency between work days and free days could be shown and a method to cancel out work day effects has been proposed (Roenneberg *et al*, 2004b, Supplemental data). Also, results of the pilot study indicated an association between chronotype and the average time spent outside under natural daylight conditions. Furthermore, a systematic delay of chronotype during adolescence has been identified (Roenneberg *et al*, 2004b). The MCTQ additionally asks for age, height, weight, and address (all voluntary). This allows the investigation of associations between chronotype and these factors and might lead to conclusions for further experiments.

1.7. Aims of this work

In order to introduce the MCTQ as a valid tool in human circadian research, its capability to assess actual sleep times is scrutenized using respective sleep log data. Furthermore, underlying structures within the set of questions of the MCTQ are investigated to refine the definition of chronotype and to improve the MCTQ by revealing and eliminating potential redundencies. Finally, factors that potentially influence chronotype are quantified and related to each other.

The aim of this work is the validation and improvement of the MCTQ and an in depth phenotypisation of chronotypes necessary for quantitative genetic analyses of underlying genes.

2. Material & Methods

2.1. Munich ChronoType Questionnaire (MCT-Q)

2.1.1. Properties of the Munich ChronoType Questionnaire

In this study, the Munich ChronoType Questionnaire (MCTQ, Roenneberg *et al*, 2003; Appendix 2) is the major tool for collecting and assessing different chronotypes. In contrast to the commonly used Morningness-Eveningness questionnaire by Horne and Ostberg (Horne & Ostberg, 1976) it allows the quantitative evaluation of chronotype by asking simple straightforward time questions (hh:mm). The possibility to differentiate between work days and free days is an extremely important issue in assessing chronotype because work times do not fit the preferred sleep times of most people (Roenneberg *et al*, 2003). Therefore the MCTQ asks all time questions seperately for work days and for free days.

Because of the major role of light in the entrainment of other mammals it's important to investigate the influence of light on the human circadian system. The MCTQ allows the assessment of daily time spent outside under natural light conditions, again separately for work days and for free days.

Subjective self assessment of chronotype offers the possibility to relate the personal feeling of chronotype to the quantitatively calculated chronotype. Self assessment ist asked for on a scale from 0 (extreme early type) to 6 (extreme late type) for the present time, how people remember themselves as a child and as a teenager, and for the family (mother, father, siblings, and partner).

Finally, people were asked for their age, gender, height, and weight which allows to investigate their putative relationship with chronotype.

The questionnaire opens with a page informing volonteers briefly about the background of the study, confidentiality, and data handling.

2.1.2. Versions of the MCTQ

In the beginning the questionnaire has been distributed in a printed version (Appendix 2) mostly during lectures among students but also to the general population in Germany and Switzerland. In order to reach a higher number of people, the questionnaire has first been converted to an interactive WORD document containing text and dropdown fields that can be filled out and saved. This offered the possibility to distribute the questionnaire via e-mail and resulted in an electronic readout without manual entering data from the hand-written paper questionnaire. However, this method has some crucial disadvantages (internet security, ambigious entries, data handling) and was finally discarded. We, therefore, chose an alternative

way of electronic accessibility. The questionnaire was transformed into a HTML form. After filling out the questionnaire online, data are submitted simply by clicking the button “Submit”. In case of wrong entries data can be deleted by clicking the “Reset” button. Individual questionnaire data will then be sent as text file to an e-mail address dedicated only to this purpose. This procedure exhibits important features which will be further discussed in detail.

a) Accessibility and structure: The questionnaire can be reached via an internet address (www.imp-muenchen.de/?MCTQ) that leads to a page with guidelines for filling out the questionnaire. From this site people can reach additional pages to obtain further information about data protection and the background of the study (corresponding to the first page of the printed version). For an overview of the different pages see **Fig.2.1.**

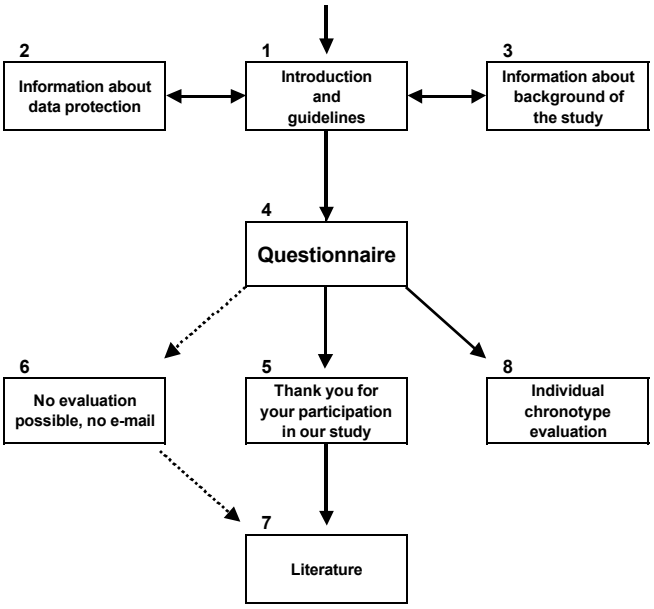


Fig.2.1.: Flow chart of the HTML pages placed before and after the questionnaire.
1-3: Introduction and information about confidentiality, data handling, data protection, and the importance of our study for public health.
4: Munich ChronoType Questionnaire, warnings are shown if entries are outside the allowed range or data necessary for the calculation of chronotype is missing. For details see text.
5+6: These pages are shown after submitting the questionnaire and announce either that the individual chronotype evaluation will be sent to the given e-mail address (5) or if an evaluation has not been possible in case of missing data (6). Both pages contain a link to a page offering the download of literature for further reading (7).
8: If sufficient data and a valid e-mail address is given, participants receive a chronotype evaluation attached to an e-mail. For details see text.

All pages are embedded in the homepage structure of the Institute of Medical Psychology, and the Typo3 content manager is used for administration. General placement of the questionnaire and Java programming was performed by dpool® internet services.

b) Security: The data given in every individual questionnaire is sent as a text file. Participants have no access to the content of the e-mail carrying the text file. A distribution of viruses, worms,… is not possible (as it was the case for the interactive WORD documents).

c) Automated control of entered data: To prevent nonsense data, Java applets are added to the HTML form. For every question, practical ranges are defined and a warning is shown if entries are below or above the defined thresholds (e.g. age between 6 and 100 years, daily time spent outdoors between 0 and 16 hours).

d) Automatic evaluation: Participants receive an automated chronotype evaluation. Depending on chronotype determined results assign to certain predefined groups based on MS_{FSc} (phase marker for chronotype, see chapter 2.4.2.) and sleep dept (difference between sleep duration on work days and average sleep duration). First, MS_{FSc} is calculated using the formula described in chapter 2.4.2. Then sleep dept accumulated during the work week (or on free days) is calculated by subtracting average sleep duration from sleep duration on work days. Following bins have been fixed:

MS_{FSc} < 2 , 2 < 2.5 , 2.5 < 3 , … , 8 < 8.5 , 8.5 < 9 , ≥ 9 (→ 17 groups)

Sleep dept (min) < -60 , -60 < -30 , -30 < 0 , 0 , 0 < 30 , 30 < 60 , ≥ 60 , no data entered for work days (→ 8 groups)

This results in a total number of 136 groups (8 sleep dept groups subdividing every MS_{FSc} group) which are displayed in respective combinations as shown on **Fig.2.2.A+B**:

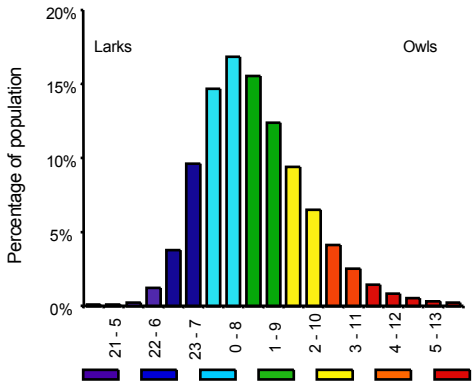


Fig.2.2.A: According to the self assessment choices, bins are summerized in 7 chronotype groups, ranging from extreme early type (violet) to extreme late type (red). Labels of the x-axis show sleep times of each chronotype group assuming an 8h sleep. In the automaticly created evaluation respective bars are indicated by an arrow.

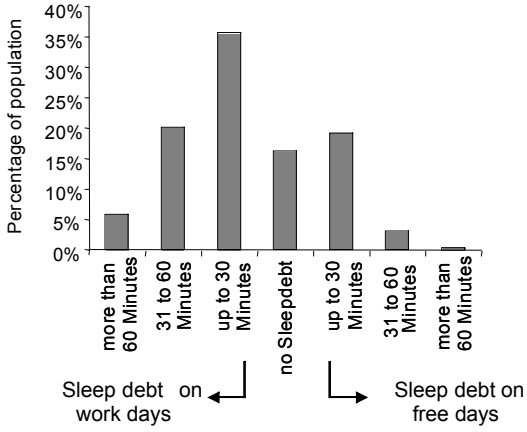


Fig.2.2.B: Groups of sleep dept accumulated on work days and on free days, respectively. Note that only about 15% of the population do not suffer from any sleep dept, neither on work days nor on free days. In the automaticly created evaluation respective bars are indicated by an arrow.

Both graphs are part of a two page PDF which is automaticly attached to an e-mail and sent to every participant who has entered an E-Mail address. Besides the graphical results a general introcuction plus chronotype specific information concerning the present knowledge about different chronotypes is given.

e) Accurate date and time of fill out: For seasonal investigations, the date of filling out is important. Information about time and date in the header of every questionnaire indicates the exact point of when the questionnaire has been sent. The data are submitted as a text file and a custom-made application, the MCTQ software (see 2.6.), can create a database by reading the text files (see g). Personal information (address) is stored separately from other data and is accessable only by the head of the Center for Chronobiology, Prof. Till Roenneberg. A unique code, consisting of date, running number, age, and sex, allows a quick going back to the original text files and is a link to the respective personal data.

f) Control of incoming data: Before text files are read be the MCTQ software, each e-mail is controlled by eye. Empty or disrupted questionnaires can so be discarded. Questionnaires sent twice or severel times can be detected by the MCTQ software and are excluded from evaluations.

g) Readability of text files: Having questionnaires sent by e-mail as text files has the advantage of an automated electronic reading in of data. The MCTQ software is able to read packages of up to 500 questionnaires in less than 5 minutes. A fast accessibility of data is so provided.

2.1.3 Available languages

The MCTQ has been translated into the following languages: English, Dutch, Italian, Russian, Greek, and Spanish. These languages are available in the printed version. Electronically, only the German, English, and Spanish versions are provided (www.imp-muenchen.de/?MCTQ), the dutch version can be reached at http://chrono.biol.rug.nl/mctq-nl.htm . An automated evaluation is provided for the German, Dutch, and English version.

2.2. Munich ChronoType Sleep Log (MCT-SL)

2.2.1. Properties of the Munich ChronoType Sleep Log

To ensure the correctness of data obtained by the MCTQ, candidates of different chronotypes were asked to keep a six week long sleep log. As the MCTQ, the sleep log asks for quantitative sleep times seperately for work days and free days. In order to obtain unambigious data we differentiate bed time and sleep onset. Furthermore, people can indicate if they needed an alarm clock to wake up and if sleep was restful. Also general comments on daily behaviour or deviations from usual sleep times (e.g. parties,…) can be given. This allows, in certain cases, to remove respective days from the evaluation and to reduce statistical bias.
Sleeplogs were made available in a printed (Appendix 4) and in an electronic version.

2.2.2. Evaluation of sleep log data

An interconnected network of EXCEL files was created to evaluate and summarize individual sleep log data. The basis is formed by an evaluation sheet that calculates sleep parameter for every single sleep log (**Fig.2.3.A+B**).

		Sleep onset		Sleep end		
Weeks		h	min	h	min	
1	Sun / Mon	24	0	7	0	1
	Mon / Tue	0	30	8	10	1
	Tue / Wed	23	58	7	10	1
	Wed / Thu	0	55	8	40	1
	Thu / Fri	20	55	7	45	1
	Fri / Sat	0	30	9	15	2
	Sat / Sun	1	45	9	25	2
2	Sun / Mon	23	45	6	45	1
	Mon / Tue	23	50	8	0	1
	Tue / Wed	0	30	7	50	1
	Wed / Thu	0	25	8	20	1
	Thu / Fri	4	35	10	20	2
	Fri / Sat	1	50	11	0	2
	Sat / Sun	2	10	9	30	2
3	Sun / Mon	0	8	8	10	1
	Mon / Tue	0	30	8	10	1
	Tue / Wed	0	5	7	45	1
	Wed / Thu	0	20	7	30	1
	Thu / Fri	20	40	7	22	1
	Fri / Sat	0	30	9	0	2
	Sat / Sun	24	0	10	0	0
4	Sun / Mon	23	40	8	0	2
	Mon / Tue	23	30	8	20	2
	Tue / Wed	0	53	8	0	1
	Wed / Thu	23	40	8	10	1
	Thu / Fri	23	30	8	56	1
	Fri / Sat	1	15	8	50	1
	Sat / Sun	0	23	9	0	2
5	Sun / Mon	0	30	6	45	2
	Mon / Tue	23	30	8	30	2
	Tue / Wed	22	30	8	0	2
	Wed / Thu	23	30	8	0	2
	Thu / Fri	24	0	8	10	2
	Fri / Sat	2	45	8	0	2
	Sat / Sun	23	0	8	20	2
6	Sun / Mon	23	38	8	10	2
	Mon / Tue	23	35	8	50	2
	Tue / Wed	23	52	8	25	1
	Wed / Thu	4	43	10	3	1
	Thu / Fri	23	50	8	0	1
	Fri / Sat	3	10	8	20	1
	Sat / Sun	2	0	10	50	2

	All days		Work days		Free days	
	ø	StDev	ø	StDev	ø	StDev
Sleep onset	0.42	1.58	0.25	1.64	0.62	1.52
Sleep end	8.41	0.96	8.06	0.71	8.81	1.07
Sleep duration	7.99	1.28	7.82	1.34	8.19	1.22
Mid-sleep	4.41	1.14	4.15	1.07	4.72	1.16
	MS_F		MS_{FSC}			
	4.72		4.62			

Fig.2.3.A: Results calculated from individual sleep log data shown in Fig.2.3a. Average sleep onset, sleep offset, sleep duration, and mid-sleep (see 2.4.) is calculated for work days, free days, and all days, respectively. Standard deviations are given for every value.

Fig.2.3.B: An evaluation sheet containing a randomly chosen dataset. Data are entered in hours and minutes for onset and offset of sleep. For each day it has to be indicated if it is a work day (1) or a free day (2) or if the day is removed from evaluation (0). This is the case for single outliers or if comments indicate non regular sleeping habits (e.g. party, illness,…). Values of minutes are transformed into metrical numbers, e.g. 4.5 h then equals 4:30h.

Ten evaluation sheets are integrated into one EXCEL file. Every person that has been chosen to participate (extreme chronotypes) has a certain position in the file network, depending on the number given. **Fig.2.4.** shows graphically the different steps from raw sleep log data to a matrix with rows reflecting extreme candidates and columns reflecting sleep parameter.

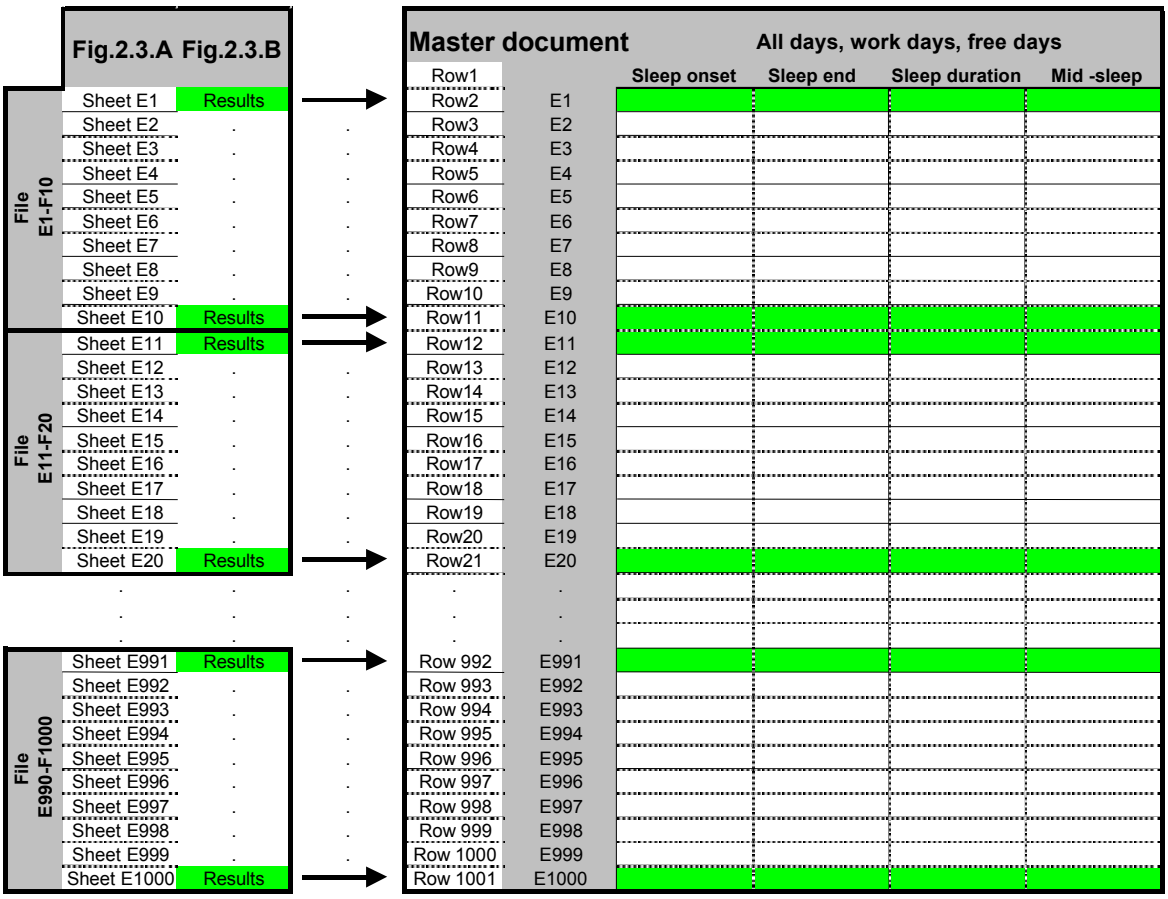


Fig.2.4.: Interconnected EXCEL sheets for the evaluation of sleep log data. Each individual receives a special code number consisting of a candidate number and the serial MCTQ number (e.g.E743-25679, L137-5137, or C20-28456, E for early types, L for the late types, C for controls). Every potential candidate is supplied with an evaluation sheet (e.g. E1 to E1000), regardless of a participation. Results are then summarized in the master document by referring to respective cells in the evaluation sheet creating a data matrix for further evaluations (standard deviations are not shown for optical reasons). In case of no participation, the rows remain empty and are sorted out.

2.3. Distribution of MCTQ & Sleep log

2.3.1. MCTQ

The majority of questionnaires (MCTQ) are filled out electronically via HTML form. Different strategies have been used to recruit participants:

a) In the beginning, questionaires were distributed in lectures for university students or general audiences in Germany (mainly Munich) and Switzerland (mainly Basel).
b) One of the biggest German survey institutes, TNS Emnid, very kindly sent an e-mail informing about our chronotype study to their entire e-mail pool consisting of about 50,000 individuals. People were told that the study is independent of TNS Emnid and that there will be no payment.
c) The German automobile club ADAC agreed to place a link to the online questionnaire in their e-mail newsletter that is sent to members and not-members of the club every two weeks. In total, 220,000 people received the newsletter.
d) Several reports on TV and articles in daily newspapers caught the interest of many people. TV reports were broadcasted in Germany, Switzerland, and Austria. Newspaper articles were published mainly throughout Germany, but few also in other countries like Austria, Switzerland, and the United Kingdom.
e) Besides the three main strategies, some people selectively searched ("googled") for topics like "biological rhythms" or "sleep", also the link found it's way into web pages that announce miscellaneous services for free, in our case the evaluation of chronotype.

2.3.2. Sleep log

Participants asked for further participation received a letter containing

a) information about the study, its aims, and its importance for public health,
b) an answer sheet for agreement or disagreement of further participation and availability of family members, and
c) a sleep log with guidelines for filling out correctly.

Letters were sent to all participants directly by mail. Both, for the answer sheet and for the sleep log, SAE with stamps on were provided.

Control groups were asked for further participation via e-mail first. Positive respondents received a letter without answer sheet but with an additional paper version of the MCTQ (for comparison between electronic version and paper version and test-retest-reliabilty).

In some cases, sleep logs were sent as electronic version as Excel sheet via e-mail.

2.4. Definition of the phase marker for chronotype and classification of extreme chronotypes

2.4.1. List of abbreviations (the list is also available as fold-out on the last page)

MCTQ questions an derivatives:

MS_W Mid-sleep on work days
MS_F Mid-sleep on free days
MS_{FSc} Mid-sleep on free days corrected for sleep dept on work days

SLD_W Sleep duration on work days
SLD_F Sleep duration on free days
$SLD_Ø$ Average sleep duration

BT_W Bed time on work days
BT_F Bed time on free days

SO_W Sleep onset on work days
SO_F Sleep onset on free days

SE_W Sleep end on work days
SE_F Sleep end on free days

DIP_W Mid-day dip on work days
DIP_F Mid-day dip on free days

IWT_W Immediate wake up time on work days
IWT_F Immediate wake up time on free days

FA_W Fully awake on work days
FA_F Fully awake on free days

Predictors:

BMI Body mass index
ØDOLE Average daily outside light exposure
PHOTO Photoperiod, Time of the year
LAT Latitude
POR Place of residence

Predictors are always written in capital letters

Sub-groups:

Females & Males
Age ≤20 (years) & Age >20 (years)
Age ≤30 (years) & Age >30 (years)
MS_{FSc} ≤4.28 & MS_{FSc} >4.28
Work days & Free days

Sub-groups are written with a capital

2.4.2. Mid-sleep on free days and its correction

a) Mid-sleep on free days (MS_F)

The daily repeating change of sleep and wakefulness is the most prominent behavioural output of the human circadian clock and it can be assessed by simply asking people for their usual sleep times. The pilot study on human chronotypes, Roenneberg *et al* (2003) used mid-sleep on free days as phase marker for chronotype. Mid-sleep is the exact middle between sleep onset (bed time + time it takes to fall asleep) and sleep end. **Fig. 2.5.** shows some examples of hypothetical chronotypes.

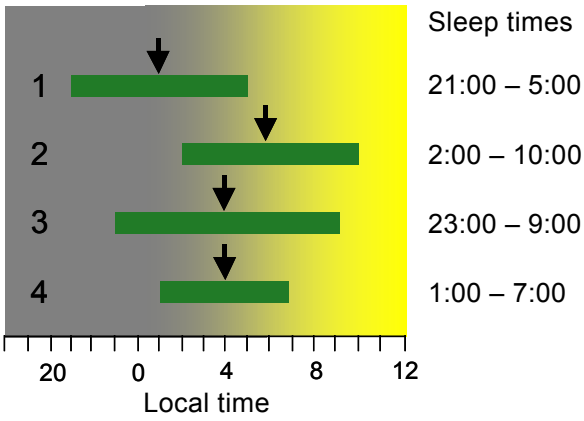


Fig.2.5.: Four hypothetical examples of sleep times on days without obligations (free days), represented by green bars. Black arrows indicate mid sleep on free days. Person 1 sleeps from 21:00 to 5:00. This results in a mid sleep of 1:00. Person 2 exhibits a mid sleep of 6:00 (sleeping from 2:00 to 10:00). Both sleep for 8h. Person 3 sleeps from 23:00 to 9:00, person 4 sleeps from 1:00 to 7:00. The first sleeps for 10h, the latter for 6h, however, both are of the same chronotype. Mid sleep on free days (MS_F) based chronotype does not depend on the duration of sleep.

The distribution of midsleep on free days (MS_F) obtained from about 15,000 qestionnaires, is shown in **Fig.2.6.**

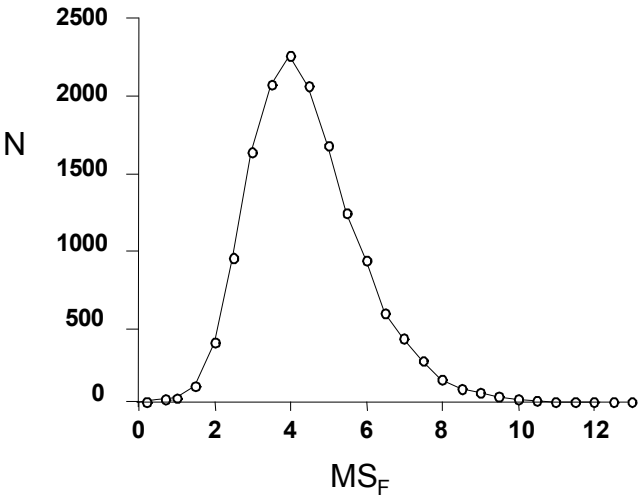


Fig.2.6.: Distribution of mid sleep on free days (MS_F, n=15,165).

b) Mid-sleep on free days corrected for differences between sleep duration on free days and sleep duration on work days (MS_{FSc}, Roenneberg *et al*, 2004b supplemental data).

With increasing MS_F, sleep duration on work days decreases while sleep duration on free days increases. Late chronotypes, therefore, accumulate sleep dept during the work week for which they compensate on free days by sleeping in (**Fig.2.7.**, Roenneberg *et al*, 2003; Valdez *et al*, 1996).

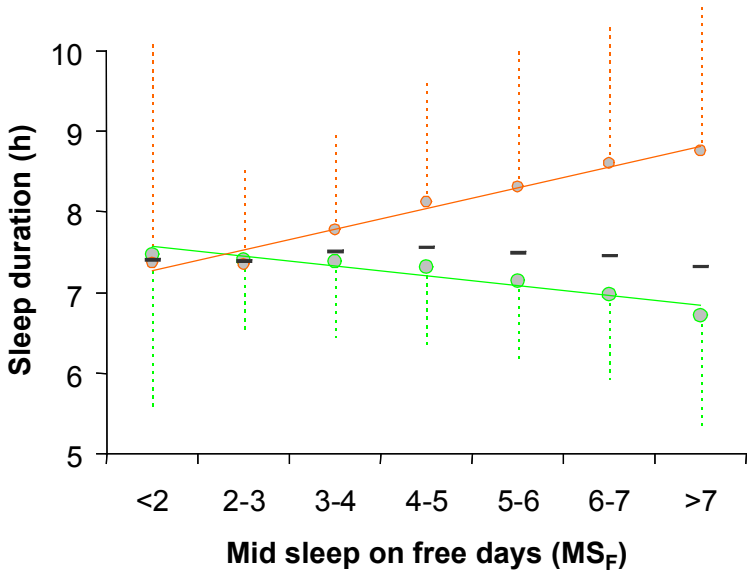


Fig.2.7.: Data presented by Roenneberg at al (2003) redrawn for 29,500 questionnaires. Linear regression for binned/raw data: work days (green): r=0.95/-0.18 (p<0.001), free days (orange): r=0.98/0.22 (p<0.001). Black bars show average sleep duration (5 times work days plus 2 times free days divided by 7).

While late types suffer from work schedules being too early, extreme early types suffer from social constraints of staying up late on free days (group MS_F<2).

Because compensating for sleep dept by sleeping in on free days delays MS_F, a correction for the descrepency between sleep duration on free days and average sleep duration is performed (**Fig.2.8.**)

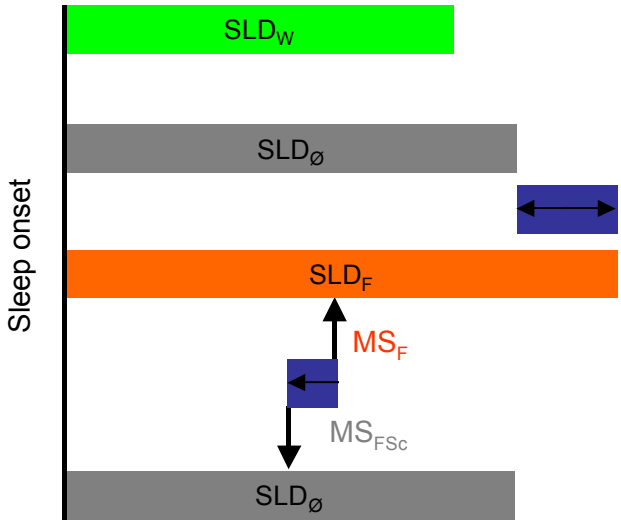


Fig.2.8.: The difference between average sleep duration and sleep duration on free days is shown as blue bar. Half of the difference between sleep duration on free days and average sleep duration is subtracted from MS_F. The figure shows an example with sleep dept on work days. In contrast, extreme early chronotypes suffer from sleep dept on free days.

Following formula is used to perform the correction as described in **Fig.2.8.**:

$$\mathbf{MS_{FSc} = MS_F - 0.5 \times (SLD_F - SLD_{Ø})} \text{ with } SLD_{Ø} = (5 \times SLD_W + 5 \times SLD_F)/7$$

In case of sleep dept on work days, the value within parenthesis is positiv and is subtracted from MS_F. If average sleep duration is longer than sleep duration on free days, the value within parenthesis is negative.

2.4.3. Use of MS_F and MS_{FSc} and classification of extreme chronotypes

Whether MS_F or MS_{FSc} is used depends on the question. For the comparison of MCTQ data and the respective sleep log, one has to use MS_F, because the major question is whether MCTQ is capable of assessing (the un-corrected) sleeping behaviour on free and work days correctly. Using MS_{FSc} is problematic due to the fact that for individual sleep logs the ratio of work days and free days can differ considerably (ranging from two free days within six weeks to six weeks of free days) while the correction uses the ratio 5 x work days / 2 x free days per week.

On the population level MS_{FSc} is used for inter-individual comparison and classification of chronotype in general. If not mentioned otherwise, MS_{FSc} is used for classification of chronotype. Based on the distribution of MS_{FSc}, obtained from about 15,000 qestionnaires, we defined 2.5% at each end of the distribution as extreme chronotypes (**Fig.2.9.**, grey areas).: Earlies ($MS_{FSc} \leq 2.17$), controls ($2.17 < MS_{FSc} \leq 7.25$), and lates ($MS_{FSc} > 7.25$).

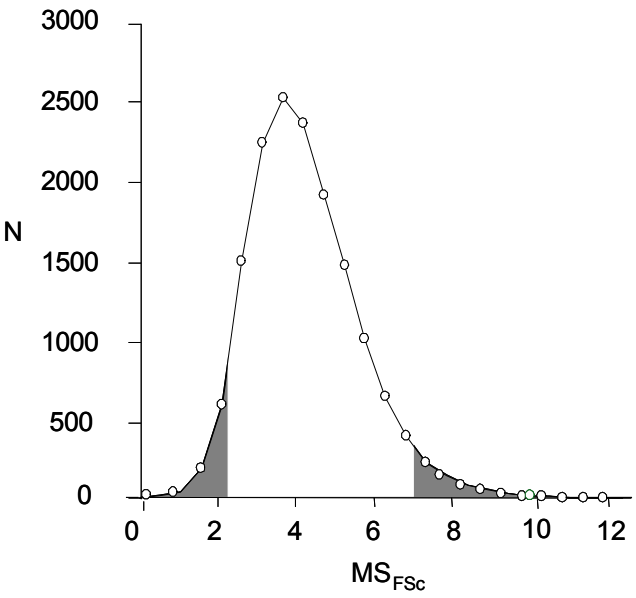


Fig.2.9.: Distribution of MS_{FSc} (n=15,165). Extreme early ($MS_{FSc} \leq 2.17$) and late ($MS_{FSc} > 7.25$) chronotypes are shown as grey areas.

2.5. Statistical methods and models

Statistical methods like correlation, t-Test, non-parametric tests or ANOVA will not be described here. The conditions of all analyses presented are described in detail in the respective chapter. Here, only the different steps within SPSS are shown in order to make the procedures understandable. All analyses were performed using the German version of SPSS 12.0 and 13.0 for Windows. Procedures were performed following recommendations from Backhaus *et al* (2003), Bortz (1999), Field (2002) and Zöfel (2001).

Factor Analysis (chapter 3.3.4.)

1. Menu path: Analyze → Data reduction → Factoranalysis
2. Transfer all variables to be included to the box labelled *Variables* (see 3.3.5.1.)
3. Select dialog box *Descriptives* and choose all options

→ By default, Principal Component Analysis is selected and all factors with Eigenvalues >1 will be extracted. No rotation is required (see 3.3.5.1.).

4. Click *OK* button to run analysis

Multiple Regression (chapter 3.4.2.)

1. Menu path: Analysis → Regression → Linear …
2. Transfer either MS_{FSc} or $SLD_{Ø}$ to the box labelled *Dependent variable*
3. Transfer Age and Gender to the box labelled *Independents* and choose the *Method: Stepwise* and click the button *Next* to open a new block
4. Transfer Light, Photoperiod, and Latitude to *Block2*, choose the *Method: Stepwise* and click the button *Next* to open a new block
5. Transfer City and BMI to *Block3*, choose the *Method: Stepwise*
6. Select dialog box *Statistics* and choose all options.
7. Select dialog box *Plots* and transfer **ZPRED* to the box labelled *X* and **ZRES* to the box labelled *Y*. Choose the option *Histogram* and *Normal probability plot*.
8. Click *OK* button to run analysis

→ Run analysis also with MS_W & MS_F instead of MS_{FSc} and with SLD_W & SLD_F instead of $SLD_{Ø}$ (chapter 3.4.3.4.)

→ Run analysis separately for Females & Males and Age ≤30 years & Age >30 years (chapter 3.4.3.5.). **Important:** Remove the independent variables Gender and Age from the respective analysis!

Analysis of Covariance (ANCOVA, chapter 3.4.3.**)**

1. Menu path: Analyze → General Linear Model → Univariate
2. Transfer either MS_{FSc} or $SLD_{Ø}$ to the box labelled *Dependent variable*
3. Transfer Gender, Age (categories), Photoperiod, and City to the box labelled *Fixed Factors*
4. Select dialog box *Contrasts* and choose *Simple (first Reference Category)* for every fixed factor.
5. Select dialog box *Options* and transfer all Factors and Factor interactions to the box labelled *Display Means for…* . Select *Compare main effects* and *Sidak* from the drop-down field. Choose following options: *Descriptive statistics*, *Parameter estimates*, *Homogeneity tests*, *Lack of fit*, and *General estimable function*.
6. Click *OK* button to run analysis

Note: Not all outcomes obtained by the different functions selected will be presented in the respective chapter.

2.6. Software

MS Office 2000 software package (Mac OSX, Windows XP, *Microsoft*)
- **Excel** for editing and general processing of all manual data entries, outputs from MCTQ software and SPSS. All graphs, regression lines, and t-Tests were performed using Excel.
- **PowerPoint** for the creation of graphs, figures and presentations
- **Word** for writing what you now read

StatistiXL 1.5 (Windows XP; Add-in for MS Excel, *Alan R. Roberts and Philip C. Withers*) provides all common basic and multivariat statistical methods for data evaluation within MS Excel. Outputs can directly be used for further analysis or graphical presentation.

SPSS (version **12.0** and **13.0** for Windows, *SPSS Inc.*) is the most commonly used statistical application. Datasets prepared with MS Excel can be imported as text files and output files can be exported as MS Excel files (only Windows version) for graphical presentation or further analysis.

MCTQ software (Mac OS9; *Till Roenneberg*) has been especially written for processing and storing data obtained by the Munich ChronoType Quesionnaire and underwent various modifications and updates. The basic function of the application is handling the continously growing database of questionnaires. Data obtained via the HTML online form (see 2.1.2.) are imported and supplied with a serial number and a code consisting of date, serial number for each date, age, gender.
Basic statistical methods such as distributions and correlations can be performed. All results are exportable as *KaleidaGraph* files for further use, also the entire database, can be saved as tab-delimited text file. Personal information of participants can only be accessed by password. Only in case of the identification of interesting chronotypes, name and address are needed for contact.

Typo3 (HTML content manager, *Kasper Skårhøj*) provides a platform for hierarchically organized HTML pages. The structure of pages shown in 2.1.2a was created within the existing homepage of the centre for chronobiology.

3. Results

3.1. Data collection

3.1.1. MCTQ

By using a publicly accessable online version of the MCTQ (2.1.2.) and with different strategies of announcement (2.3.1.) a total number of over 34,000 questionnaires could be obtained. This is the largest database about human circadian behaviour so far. Due to different opportunities of announcemant and distribution the flow of questionnaires greatly varied. **Fig.3.1.** shows the number of incoming questionnaires per month.

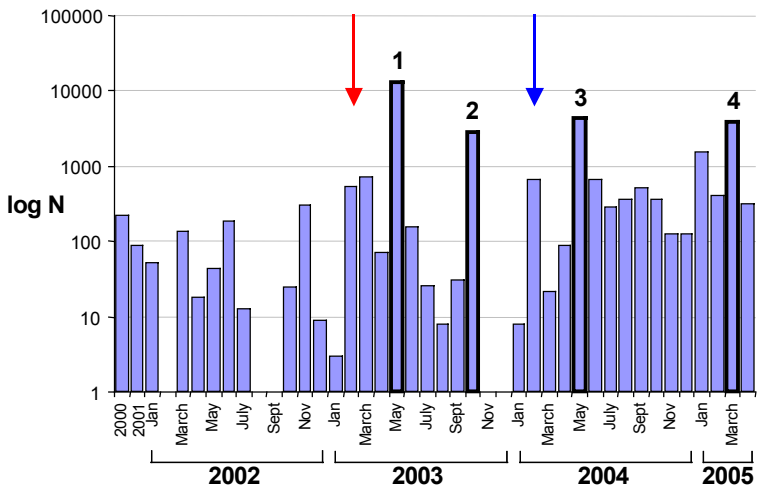


Fig.3.1.: Monthly rate of incoming MCTQ (logarithmic scale). The years 2000 and 2001 are shown in total due to the low numbers received. The project started January 2003 and incoming data is graphed until March 2005. Red arrow indicates time of the first strategy used (see 2.3.1.). Bars with bold borders show peaks of incoming questionnaires:
1: Emnid (2.3.1b), 13,300 questionnaires
2+4: TV reports (2.3.1d), 2800 and 3900 questionnaires, respectively
3: ADAC (2.3.1c), 4400 questionnaires
From about the begin of 2004, unregular articles about chronobiology in daily and weekly newspapers contained a link to our questionnaire (2.3.1d, blue arrow). A more or less constant flow of questionnaires is maintained since then.

The majority of questionnaires has been filled out in Germany, Austria (507), and the German speaking part of Switzerland (804). A small sample of 179 questionnaires is available from the United Kingdom. Other countries (Italy, India, France, …) put together contributed between 50-100 questionnaires.

3.1.2. Sleep log (MCT-SL)

A major aim of the study was to collect high numbers of extreme chronotypes for genetic analysis. Most sleep logs, therefore, belong to either the early or the late group of chronotypes. Recruiting of participants was performed as described in 2.3. We judged every person as extreme chronotype if MS_{FSc} was above or below the respective threshold (see 2.4.3.).

A total number of 672 sleep logs were obtained so far (based on 29,000 subjects in our database). Most sleep logs returned about six weeks after having been sent to participants. Nevertheless, a delay of several weeks or even months occurred in at least one third of the cases. For details and a summary of numbers see Table 3.1.

	Early	Control	Late	Sum
Contacted	774	670	1178	2622
Positive reply	309	262	448	1019
% of contacted	39.9	39.1	38.0	38.9
Sleep logs	259	128	285	672
% of positive replies	83.8	48.9	63.6	65.9

Table 3.1.: Numbers of sleep logs obtained. Extreme chronotypes (early and late) and controls were contacted as described in 2.3.2. Controls were randomly chosen individuals with MS_{FSc} between 2.17 and 7.25. For every group about the same percentage replied positively. Middle rows (positive replies) show the number of individuals that positively responded via answer sheet (extremes) or via e-Mail (controls).
The actual number of filled out sleep logs (lower rows) differs between groups. About 84% of the earlies but only about 64% of the lates sent back a filled out sleep log.

3.2. Validation of the Munich Chronotype Questionnaire

3.2.1. Test – Retest – Reliability

Note: The list of abbreviations is available as fold-out on the last page

A random sample of 521 people (control group) that filled out the electronic version in August and September 2004 was contacted via e-mail mid January 2005. In total, 206 people replied positively and subsequently received a letter containing a paper version of the MCTQ. By the time of evaluation, 101 paper questionnaires were available.

Sub-grouping for Gender and Age: Differences in circadian behaviour have been reported for gender (Adan & Natale, 2002) and Age (Yoon *et al*, 2003; Roenneberg *et al*, 2004b). To see if reliability is also dependent on these factors, results of regressions of both time points (Aug/Sept 2004 and January 2005) are plotted for the whole sample and separately for Gender and Age (≤30 years and >30 years) for MS_F, SO_W, SE_W, SO_F, SE_F, and ØDOLE.
Note: The term *Gender* will be used to describe differences between females and males regardless of a biological or sociological origin.

Sub-grouping for chronotype: It has been reported that circadian preference can result in different behaviour patterns (Giannotti *et al*, 2002). To test if this applies for the MCTQ chronotype assessment, reliability is controlled for the early and the late half of the sample. Chronotype groups are determined using MS_{FSc} from the electronic version. Individuals with MS_{FSc} below 4.28 (which is the average MS_{FSc} calculated from 29,500 electronic questionnaires) belong to the early group, the late group consists of individuals with MS_{FSc} equal or higher 4.28. If not mentioned otherwise, this average value will be used in all further analyses.

Differences and associations: Exept for ØDOLE, there is not significant difference between MCTQ parameters from the two different time points of assessment (paired t-Test: $p>0.027$, Bonferoni corrected level of significance: $p=0.0012$). ØDOLE is not significantly different for Males, Age≤30, and $MS_{FSc} \geq 4.28$ (**Table 3.2.**)

All correlations are highly significant ($p<0.001$, Bonferoni corrected level of significance: $p=0.0012$) exept SE_F of Age ≤30 ($p=0.002$). ØDOLE is not significant for Age≤30 ($p=0.363$) and $MS_{FSc} \geq 4.28$ ($p=0.01$). Only for ØDOLE, regression lines deviate greatly from the 1:1 ratio line with slopes <0.5; also, correlations are weaker compared to other parameters. This is probably due to the fact that people are more outside in summer than in winter.

	r	p (r)	Regression	p (paired t-Test)	n
MS_F					
All	0.882	<0.001	y=0.907x+0.437	0.977	96
Females	0.895	<0.001	y=0.965x+0.175	0.895	66
Males	0.858	<0.001	y=0.783x+0.802	0.858	30
≤30 years	0.778	<0.001	y=0.850x+0.778	0.148	25
>30 years	0.911	<0.001	y=0.965x+0.204	0.198	71
MS_{FSc} <4.28	0.793	<0.001	y=0.828x+0.641	0.885	69
MS_{FSc} ≥4.28	0.672	<0.001	y=0.801x+1.032	0.821	27
SO_W					
All	0.881	<0.001	y=0.864x-0.187	0.061	96
Females	0.866	<0.001	y=0.824x-0.217	0.096	63
Males	0.910	<0.001	y=0.984x-0.060	0.543	29
≤30 years	0.833	<0.001	y=0.754x-0.300	0.167	25
>30 years	0.903	<0.001	y=0.924x-0.124	0.191	66
MS_{FSc} <4.28	0.805	<0.001	y=0.835x-0.289	0.029	64
MS_{FSc} ≥4.28	0.753	<0.001	y=0.637x-0.007	0.670	27
SE_W					
All	0.889	<0.001	y=0.901x+0.646	0.682	92
Females	0.909	<0.001	y=0.866x+0.947	0.652	63
Males	0.775	<0.001	y=0.893x+0.570	0.234	29
≤30 years	0.816	<0.001	y=0.787x+1.363	0.371	25
>30 years	0.938	<0.001	y=1.001x+0.008	0.573	66
MS_{FSc} <4.28	0.926	<0.001	y=1.069x-0.406	0.331	67
MS_{FSc} ≥4.28	0.804	<0.001	y=0.779x+1.484	0.225	27
SO_F					
All	0.886	<0.001	y=0.808x-0.035	0.221	96
Females	0.875	<0.001	y=0.745x+0.001	0.398	66
Males	0.926	<0.001	y=0.987x-0.081	0.268	30
≤30 years	0.851	<0.001	y=0.769x-0.137	0.027	25
>30 years	0.901	<0.001	y=0.850x+0.010	0.840	71
MS_{FSc} <4.28	0.829	<0.001	y=0.821x-0.050	0.820	69
MS_{FSc} ≥4.28	0.560	0.003	y=0.538x+0.403	0.029	27
SE_F					
All	0.777	<0.001	y=0.773x+1.907	0.489	96
Females	0.821	<0.001	y=0.891x+1.006	0.262	66
Males	0.702	<0.001	y=0.568x+3.398	0.813	30
≤30 years	0.608	0.002	y=0.515x+4.182	0.684	25
>30 years	0.821	<0.001	y=0.887x+1.012	0.208	71
MS_{FSc} <4.28	0.722	<0.001	y=0.701x+2.313	0.971	69
MS_{FSc} ≥4.28	0.650	<0.001	y=0.581x+4.061	0.312	29
ADOLE					
All	0.477	<0.001	y=0.394x+0.737	< 0.001	93
Females	0.466	<0.001	y=0.453x+0.543	< 0.001	65
Males	0.456	0.018	y=0.313x+1.102	0.009	28
≤30 years	0.189	0.363	y=0.214x+1.403	0.174	25
>30 years	0.604	<0.001	y=0.439x+0.538	< 0.001	68
MS_{FSc} <4.28	0.511	<0.001	y=0.398x+0.736	< 0.001	67
MS_{FSc} ≥4.28	0.409	0.039	y=0.361x+0.772	0.011	27

Table 3.2.: Correlation coefficient (r), equation of regression line, paired t-Test, and n of MS_F, SO_W, SE_W, SO_F, SE_F, and ADOLE for different groups (total sample, Females, Males, Age ≤ 30, Age>30, MS_{FSc} <4.28, and MS_{FSc} ≥4.28. The respective graphs are shown in **Fig.3.2.** (total sample, Females, Males, Age ≤ 30, Age>30) and **Fig.3.3.** (MS_{FSc} <4.28, and MS_{FSc} ≥4.28).

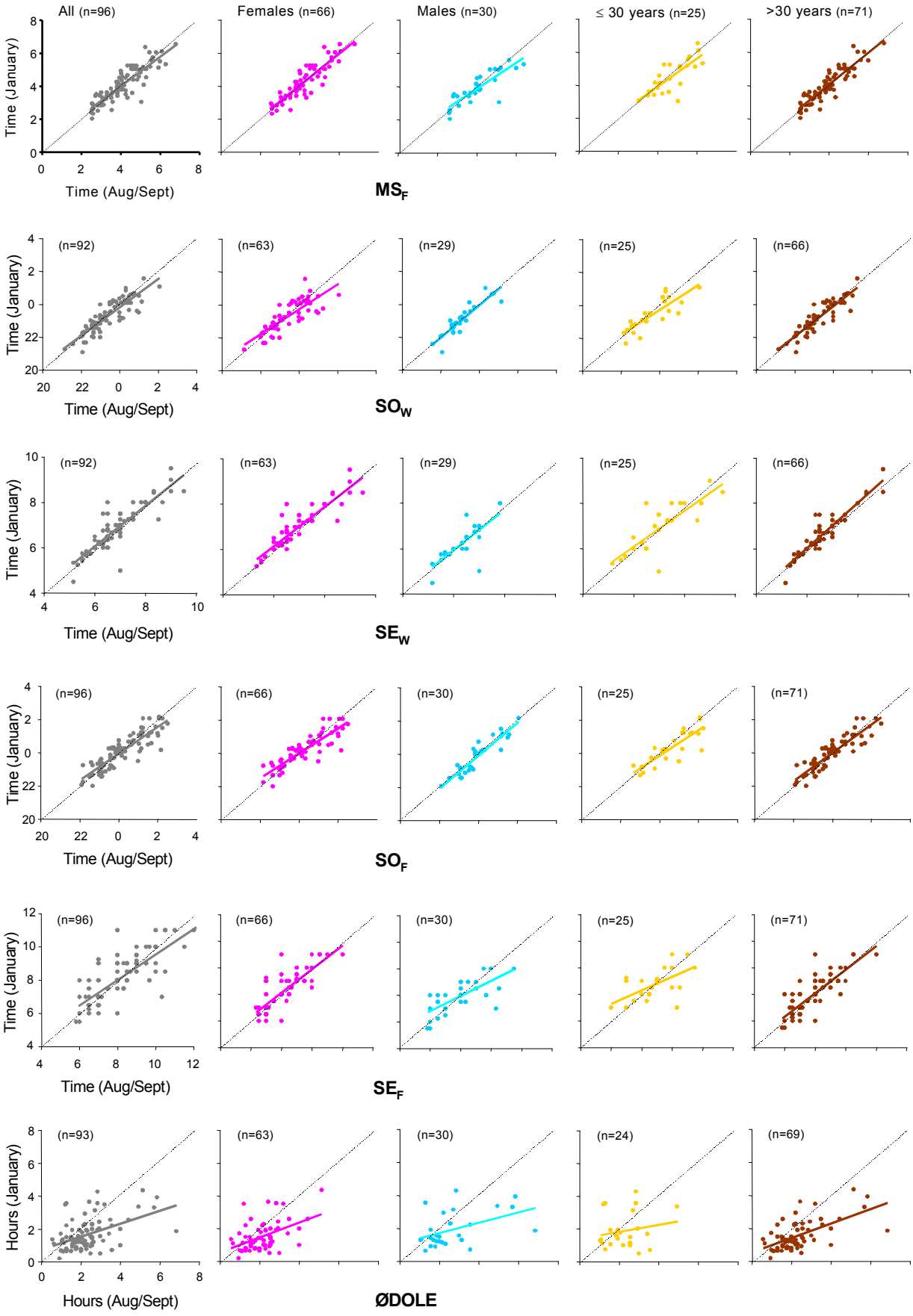


Fig.3.2.: Regression graphs for the MCTQ filled out Aug/Sept 2004 and January 2005 for the total sample (grey), Females (pink), Males (blue), Age ≤ 30 (gold), Age>30 (brown). Two outlyers were removed from ØDOLE. For details see text.

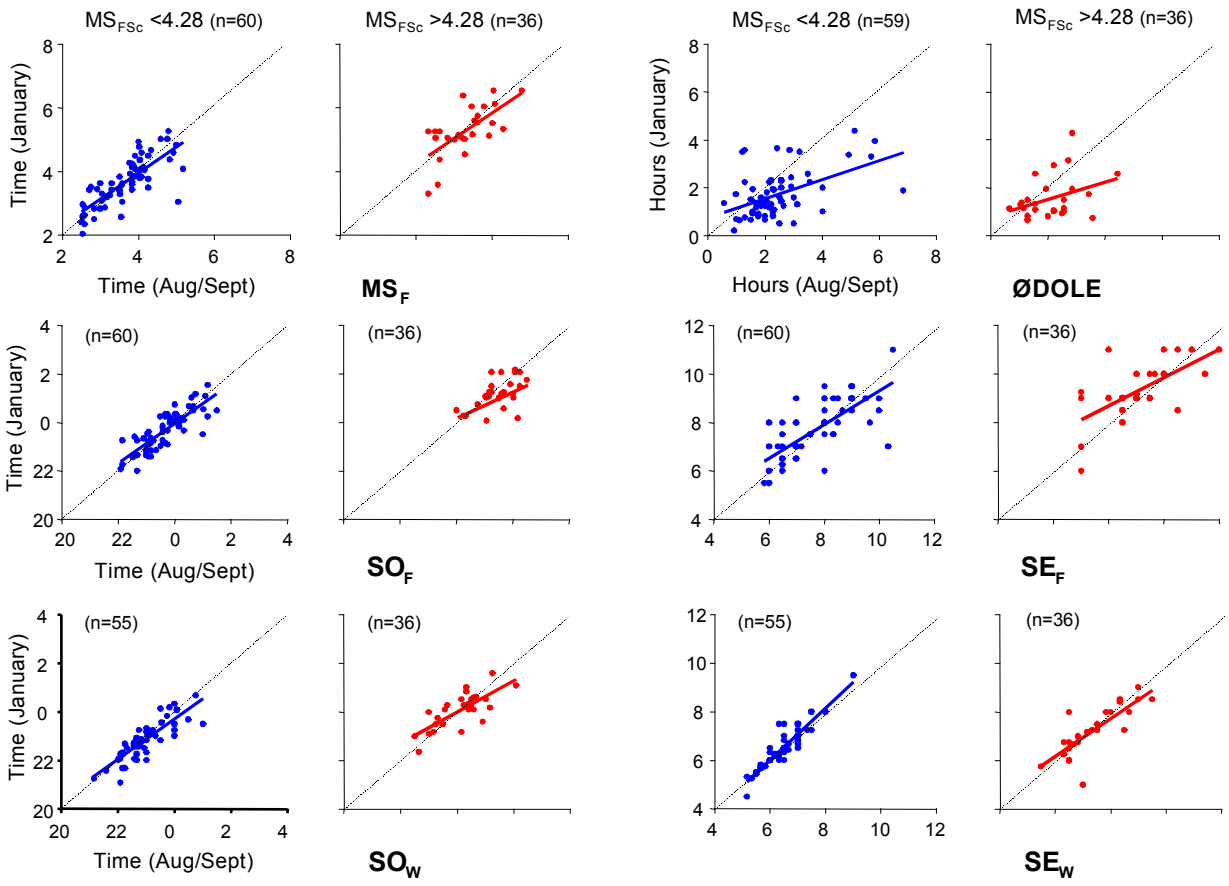


Fig.3.3.: Regression graphs for the MCTQ filled out Aug/Sept 2004 and January 2005 for MS_{FSc} <4.28 (blue), and MS_{FSc} ≥4.28 (red). Two outlyers were removed from ØDOLE. For details see text.

In general, most constant values are apparent for Age >30 throughout all parameters whereas Age≤30 shows a slight deviation from the 1:1 line. MS_{FSc} < 4.28 correlates better than MS_{FSc} ≥4.28 for every parameter. For ØDOLE, correlations of MS_{FSc} > 4.28, Age≤30, and Males are not significant after Bonferoni correction. Graphs of regression analysis are shown in **Fig.3.2.** (total sample, Females, Males, Age≤30, Age>30) and **Fig.3.3.** (MS_{FSc} <4.28, and MS_{FSc} ≥4.28)

Sleep times show a high consistency over time even if taking in account that sleep times are generally different between the summer and winter months with advanced chronotype in summer (see chapter 3.4.). Differences between groups (Gender, Age, Chronotype) are likely due to different schedules and environmental influences they are exposed to: e.g. females are more often influenced by their children, younger ages are often in a less stable employment or are students, and late types have to adapt to relatively early working schedules. Although there are slight differences between groups, retesting comes up with very similar results for parameters essential for determination of MS_{FSc}. A sufficient reliability is assumed for MCTQ as tool for the quantitative assessment of chronotype.

A random sample of people (n=147, control group) that filled out the MCTQ within three days (January 2005) was contacted via e-mail. Participants that replied positively within a week (n=43) received a letter containing a paper version of the MCTQ. They were explicitly advised not to remember what they filled out in the electronic version. The maximum time span between both dates of filling out time of the MCTQ was set to three weeks (assessed by data entered on paper version). Within this time a total number of 15 questionnaires were sent back.

All results are highly significant ($p<0.001$) except SE_F ($p=0.033$, $p_{Sig}=0.002$ after Bonferoni correction). MS_F shows the best similarity to the 1:1 ratio with a slope close to 1 and intercept near the origin. Paired t-Test indicates no significant difference between electronic and paper version ($p>0.056$, $p_{Sig}=0.002$ after Bonferoni correction). Results shown for MS_F, SO_F, SE_F, SO_W, SE_W, and ØDOLE in **Table 3.3.**, and in **Fig.3.4.** are very similar to those obtained in 3.2.1.

	r	p (r)	Regression	p (paired t-Test)	n
MSF	0.814	<0.001	y=0.963x+0.187	0.954	15
SO_W	0.911	<0.001	y=0.818x+0.220	0.162	14
SE_W	0.820	<0.001	y=0.910x+0.409	0.246	14
SO_F	0.919	<0.001	y=1.051x-0.308	0.112	15
SE_F	0.541	<0.033	y=0.597x+3.751	0.598	15
ADOLE	0.934	<0.001	y=0.962x-0.128	0.056	14

Table 3.3.: Correlation coefficient (r), equation of regression line, paired t-Test, and n of MS_F, SO_W, SE_W, SO_F, SE_F, and ØDOLE. The respective graphs are shown in **Fig.3.4.** Level of significance is Bonferoni corrected (p=0.002). See text for details.

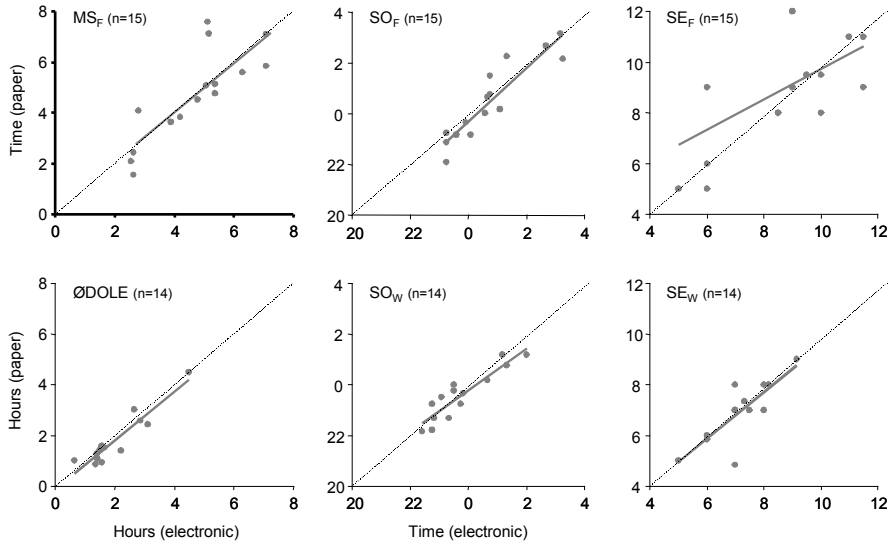


Fig.3.4.: Regression plots for MS_F, SO_W, SE_W, SO_F, SE_F, and ØDOLE. The MCTQ has been filled out in January 2005, for the first time with the online HTML form (x-axis), the second time with a paper version (y-axis).

3.2.2. Validity of the MCTQ and MS_{FSc} as phase marker for chronotype

Another essential characteristic of a questionnaire is the capability to actually measure what is intended to be measured. MCTQ assesses the average sleep times for work days and for free days with a single value. However, there can be large intra-individual variance in actual sleep times. To see if MCTQ reflects the average actual sleeping behaviour values from the MCTQ with the average values from the respective sleep log were compared.

Most sleep logs are available from individuals at both the early (n=259) and the late (n=258) end of the distribution because the primarily aim of the study was the collection of extreme chronotypes for genetic analysis. A total of 117 sleep logs from the control group (selected for $2<MS_{FSc}<7$) could be obtained until evaluation which results in 625 analysed sleep logs. **Fig.3.5.** presents the respective MS_F value of all MCTQ-sleep log pairs (represented by grey dots).

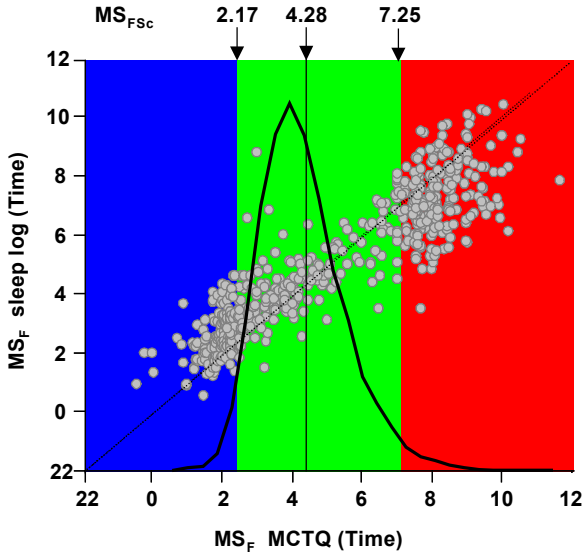


Fig.3.5.: Correlation plot of all MCTQ / sleep log combinations, shown as respective MSF values. A total of 672 sleep logs could be obtained so far (see 3.1.2.). By the time of evaluation, 625 sleep logs were available which are shown in the graph. Chronotype groups are depicted in colour (blue: extreme early, MS_{FSc} <2.17; green: normal, 2.17 < MS_{FSc} <7.25; red: extreme late; MS_{FSc} >7.25, see 2.5.1b). Black vertikal line indicates average MS_{FSc} (= 4.28).
The highes number of early types concentrate around MS_{FSc} of 2 chronotypes earlier than MS_{FSc} of 1.5 are very rare, whereas the late portion is more spread towards an open end.

When starting to select for extreme chronotypes, candidates were selected based on the threshold criteria for at least one of the mid-sleep variants (MS_F or MS_{FSc} see 2.5.). For comparison of MCTQ and sleep log, all individuals will be classified following MS_{FSc}.

Groups were analysed separately for correspondence of MCTQ data and sleep log data. **Table 3.4.** and **Fig.3.6.** show results for each group and all groups together.

	r	p (r)	Regression	Intercept with 1:1	p (paired t-Test)	n
MS_{FSc} <2.17	0.563	<0.0001	y = 0.852x + 1.009	x=6.82	<0.001	166
2.17 ≤MS_{FSc} ≤7.25	0.864	<0.0001	y = 0.720x + 1.384	x=4.94	0.688	319
MS_{FSc} >7.25	0.405	<0.0001	y = 0.581x + 2.726	x=6.51	<0.001	140
2.17 ≤MS_{FSc} ≤4.28	0.529	<0.0001	y = 0.657x + 1.558	x=4.54	<0.001	159
4.28 <MS_{FSc} ≤7.25	0.642	<0.0001	y = 0.672x + 1.754	x=5.35	<0.001	165
MS_{FSc} ≤4.28	0.662	<0.0001	y = 0.755x + 1.222	x=4.99	<0.001	330
MS_{FSc} >4.28	0.654	<0.0001	y = 0.696x + 1.659	x=5.46	<0.001	295
All	0.915	<0.0001	y = 0.745x + 1.260	x=4.94	0.755	625

Table 3.4.: All candidates who kept a sleep log were classified using MS_{FSc} from MCTQ (for classification see **Fig.3.5.**). Groups were compared for $\mathbf{MS_F}$. Bonferoni corrected level of significance: 0.0007, for details see text.

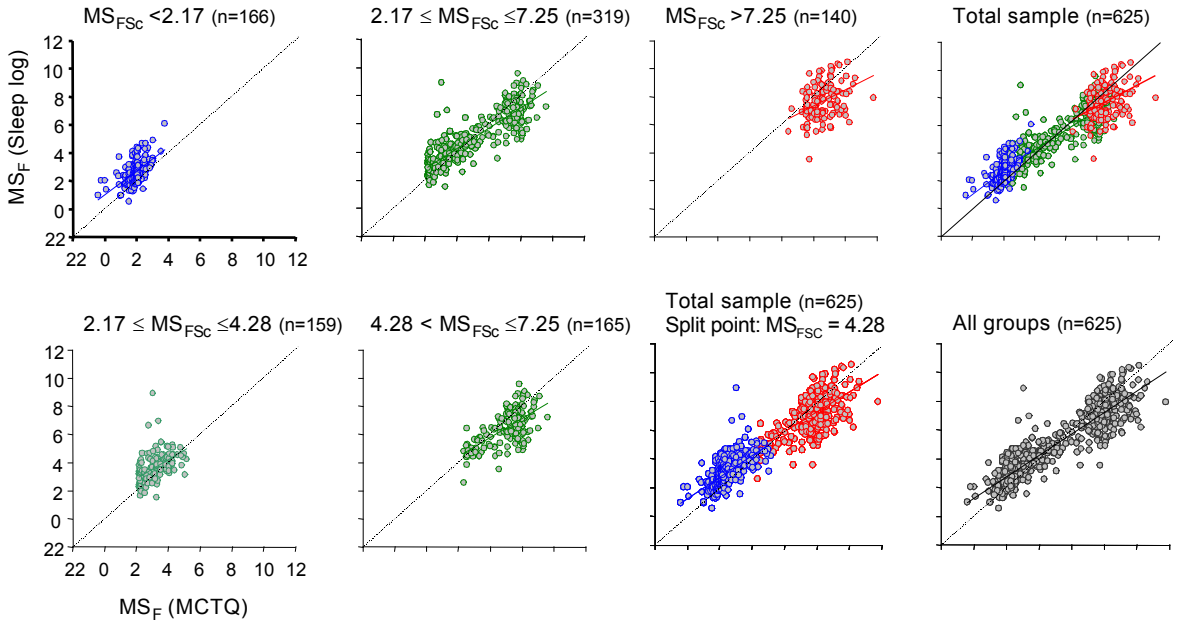


Fig.3.6: Graphs of regressions of $MS_{F\ (MCTQ)}$ and $MS_{F\ (Sleep\ log)}$ presented in **Table 3.4.** Colour coding: MS_{FSc} <2.17, 2.17 ≤MS_{FSc} ≤7.25, MS_{FSc} >7.25, 2.17 ≤MS_{FSc} ≤4.28, 4.28 <MS_{FSc} ≤7.25, MS_{FSc} ≤4.28, MS_{FSc} >4.28, all groups.

The normal group (2.17 ≤MS_{FSc} ≤7.25) shows best correlation. However, there is a tendency of overestimating chronotype towards the earlier and later part of the group, indicated by a slope of regression line of 0.720 and a point of intersection with the the 1:1 ratio line at x=4.94. On average, early type seem to assess their chronotype earlier than it actually is while late types estimate themselves later as they actually are.

When splitting up the normal group in an earlier half (2.17 ≤ MS_{FSc} ≤4.28) and a later half (4.28 < MS_{FSc} ≤7.25), different results are evident for sub-groups resembling more those of the early (MS_{FSc} <2.17) and the late (MS_{FSc} >7.25) group. Regressions show a general tendency of overestimation. Looking at regressions of the earlier (n=330) and the later (n=295)

half of the total sample (lower row, third graph from the left), a striking similarity can be observed when comparing with regression of the entire sample (lower row, right graph, n=625). Therefore, a linear increase of overestimation can be assumed with best correspondence between MCTQ and sleep log at about the average MS_{FSc} (=4.28, point of intersection with the 1:1 ratio line: x=4.94). Similar results can be observed for SO_W, SE_W, SO_F, and SE_F (**Table 3.5.**); the tendency of overestimation is also evident here with the majority of slopes between 0.5 and 0.8.

Due to overestimation, paired t-Test is significant for most groups of MS_F, SO_F, SE_F, an SO_W. No significant difference is apparent for any group of SE_W.

	MS_{FSc}	r	p (r)	Regression	Intercept with 1:1	p (paired t-Test)	n
SO_F	<2.17	0.439	<0.0001	y = 0.506x - 0.132	x=-0.27	<0.0001	166
	2.17 - 7.25	0.829	<0.0001	y = 0.743x + 0.389	x=1.51	0.009	319
	>7.25	0.278	0.0008	y = 0.389x + 2.017	x=3.30	<0.0001	140
	2.17 - 4.28	0.400	<0.0001	y = 0.523x + 0.147	x=0.31	<0.0001	164
	4.28 - 7.25	0.543	<0.0001	y = 0.665x + 0.657	x=1.90	0.009	155
	≤4.28	0.570	<0.0001	y = 0.630x + 0.849	x=2.29	<0.0001	295
	>4.28	0.278	0.0001	y = 0.389x + 2.017	x=3.30	<0.0001	140
	All	0.885	<0.0001	y = 0.746x + 0.373	x=1.47	0.010	625
SE_F	<2.17	0.524	<0.0001	y = 0.601x + 2.960	x=7.42	<0.0001	166
	2.17 - 7.25	0.823	<0.0001	y = 0.624x + 3.245	x=8.63	0.012	319
	>7.25	0.447	<0.0001	y = 0.442x + 6.053	x=10.00	<0.0001	140
	2.17 - 4.28	0.564	<0.0001	y = 0.522x + 3.829	x=8.01	<0.0001	164
	4.28 - 7.25	0.614	<0.0001	y = 0.487x + 4.962	x=9.67	<0.0001	155
	≤4.28	0.646	<0.0001	y = 0.620x + 2.990	x=7.87	<0.0001	330
	>4.28	0.615	<0.0001	y = 0.530x + 4.694	x=9.99	<0.0001	295
	All	0.886	<0.0001	y = 0.691x + 2.647	x=8.57	0.011	625
SO_W	<2.17	0.768	<0.0001	y = 0.705x – 0.114	x=-1.49	<0.0001	156
	2.17 - 7.25	0.822	<0.0001	y = 0.814x + 0.329	x=1.77	<0.0001	303
	>7.25	0.653	<0.0001	y = 0.437x + 1.378	x=2.45	0.012	131
	2.17 - 4.28	0.621	<0.0001	y = 0.717x + 0.025	x=0.09	<0.0001	150
	4.28 - 7.25	0.614	<0.0001	y = 0.667x + 0.707	x=2.12	0.0004	153
	≤4.28	0.742	<0.0001	y = 0.738x + 0.003	x=-1.14	<0.0001	306
	>4.28	0.687	<0.0001	y = 0.539x + 0.940	x=2.04	<0.0001	284
	All	0.878	<0.0001	y = 0.782x + 0.302	x=0.80	<0.0001	590
SE_W	<2.17	0.531	<0.0001	y = 0.608x + 1.971	x=5.03	0.472	156
	2.17 - 7.25	0.734	<0.0001	y = 0.796x + 1.525	x=7.48	0.101	305
	>7.25	0.517	<0.0001	y = 0.460x + 5.103	x=9.45	0.711	130
	2.17 - 4.28	0.610	<0.0001	y = 0.691x + 1.872	x=6.06	0.922	152
	4.28 - 7.25	0.555	<0.0001	y = 0.579x + 3.496	x=8.30	0.062	153
	≤4.28	0.678	<0.0001	y = 0.756x + 1.342	x=5.50	0.534	308
	>4.28	0.595	<0.0001	y = 0.558x + 3.893	x=8.81	0.159	283
	All	0.804	<0.0001	y = 0.816x + 1.339	x=7.28	0.307	591

Table 3.5.: Results, as presented in **Table 3.4.**, for SO_W, SE_W, SO_F, and SE_F. Bonferoni corrected level of significance: 0.0007, for details see text.

Three reference points can be defined for sleep phase on free can be defined with the MCTQ: MS_F, SO_F, and SE_F. MS_F has been chosen as phase reference point for chronotype because it's been reported to be the best phase anchor point for melatonin onset (Roenneberg *et al*, 2003; Terman *et al*, 2001). When comparing MCTQ data with sleep log data, MS_F shows strongest correlations and highest slopes (**Fig.3.7.**). Interceptions are closest to the origin for SO_F, highest values can be seen for SE_F. MS_F has intermediate values but relatively close to those of SO_F.

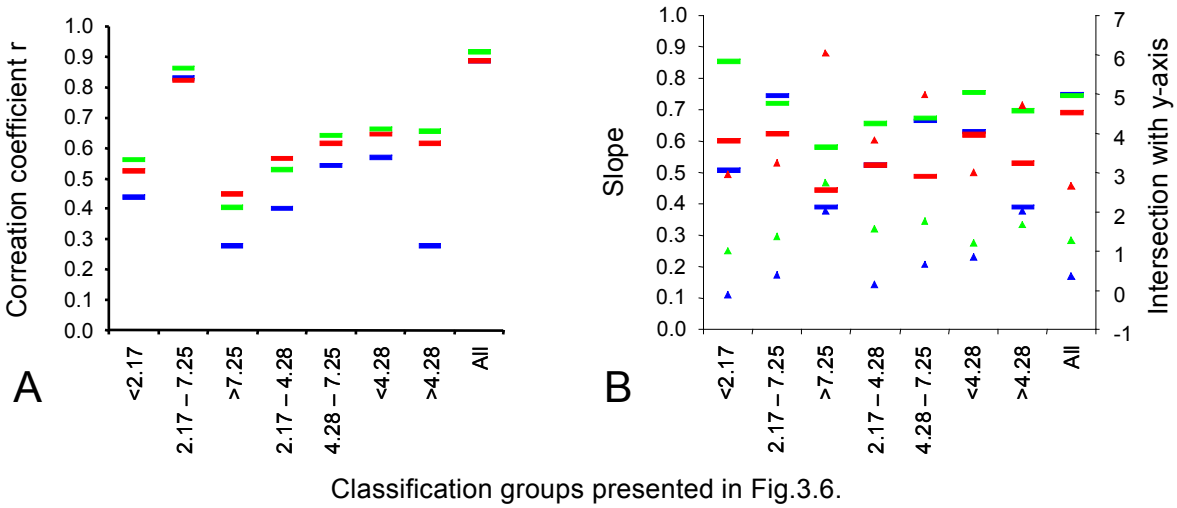


Fig.3.7.: Comparison of phase reference points for **chronotype** from groups presented in **Fig.3.6.** and **Table 3.4.** Respective values for different groups are presented as bars (blue: SO_F, red: SE_F, green: MS_F) for **correlation coefficient** (A) and **regression line** (B, bars: slope; triangles: intercept).

Inter-individual comparison of chronotypes is performed using MS_{FSc} (see 2.5.2.). Comparing MCTQ and sleep log for SO_W and SE_W results in a very similar accuracy as described for SO_F and SE_F (see **Table 3.5.**). All parameters essential for calculation of MS_{FSc} possess similar characteristics and the same tendency of overestimation towards extreme chronotypes.

Regression through all chronotype groups leads to results representative of chronotype groups analyzed separately. Comparison of Gender and Age is, therefore, carried out for the total sample and results are again very similar (**Fig.3.8.** and **Tables 3.6.+3.7.**). Thus, MS_{FSc} proofs to be a reliable parameter for quantitative assessment of chronotype.

	r	p (r)	Regression	Intercept with 1:1	p (paired t-Test)	n
Females	0.900	<0.0001	y = 0.713x + 1.374	x=4.79	0.574	290
Males	0.923	<0.0001	y = 0.765x + 1.189	x=5.05	0.344	335
Age ≤ 30	0.860	<0.0001	y = 0.685x + 1.750	x=5.56	0.0001	256
Age>30	0.912	<0.0001	y = 0.756x + 1.151	x=4.72	0.001	369

Table 3.6.: Comparison of MS_F from MCTQ and sleep log for separately for Gender and Age. Bonferoni corrected level of significance: 0.0007, for details see text.

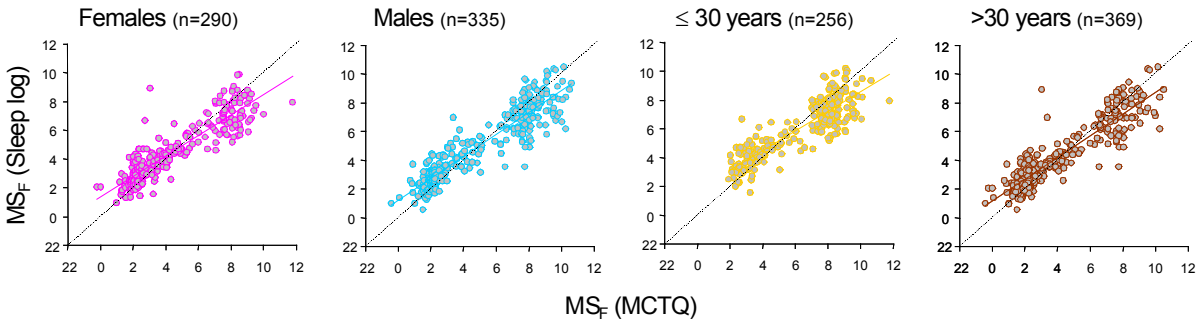


Fig.3.8.: Graphs of regressions of $MS_{F\ (MCTQ)}$ and $MS_{F\ (Sleep\ log)}$ presented in **Table 3.6.**, separately for Females, Males, Age ≤ 30, and Age>30.

		r	p (r)	Regression	Intercept with 1:1	p (paired t-Test)	n
Females	SO_F	0.863	<0.0001	y = 0.696x + 0.318	x=1.05	0.055	290
	SE_F	0.870	<0.0001	y = 0.666x + 2.857	x=8.54	0.459	290
	SO_W	0.735	<0.0001	y = 0.625x + 0.181	x=0.48	0.001	277
	SE_W	0.784	<0.0001	y = 0.802x + 1.365	x=6.88	0.945	277
Males	SO_F	0.897	<0.0001	y = 0.775x + 0.408	x=1.81	0.083	335
	SE_F	0.895	<0.0001	y = 0.706x + 2.510	x=10.00	0.006	335
	SO_W	0.848	<0.0001	y = 0.724x + 0.280	x=1.01	<0.0001	313
	SE_W	0.814	<0.0001	y = 0.822x + 1.368	x=7.67	0.206	314
Age ≤ 30	SO_F	0.825	<0.0001	y = 0.665x + 0.652	x=1.95	0.306	256
	SE_F	0.805	<0.0001	y = 0.604x + 3.780	x=9.54	<0.0001	256
	SO_W	0.781	<0.0001	y = 0.676x + 0.498	x=1.54	<0.0001	249
	SE_W	0.671	<0.0001	y = 0.615x + 3.175	x=8.24	0.005	250
Age>30	SO_F	0.894	<0.0001	y = 0.781x + 0.300	x=1.37	<0.0001	369
	SE_F	0.877	<0.0001	y = 0.691x + 2.505	x=8.11	0.155	369
	SO_W	0.804	<0.0001	y = 0.660x + 0.064	x=0.19	0.004	341
	SE_W	0.872	<0.0001	y = 0.906x + 0.503	x=5.34	0.050	341

Table 3.7.: Results as presented in **Table 3.6.** for Females, Males, Age ≤ 30, and Age>30. Bonferoni corrected level of significance: 0.0007.

3.2.3. Factors influencing precision of chronotype assessment

3.2.3.1. Variation in actual sleep times on free days

For some people, sleep times are very different between work days and free days as well as within free days and they have to declare one single time point representing a wide range of changing sleep times. The more an individual's sleep times vary the more difficult it should be to average them quickly while filling out the MCTQ. To see if assessment of chronotype depends on variance of actual sleep times, individuals with relatively constant sleep times were compared to those sleeping actually at different times almost every day. Four groups where formed based on standard deviation of MS_F calculated from sleep log data (**Table 3.8.** and **Fig.3.9.**).

StDev	r	p (r)	Regression	p (paired t-Test)	Intercept with 1:1	n_{All}	n ♀	n ♂
0.0≤0.5	0.961	<0.001	y=0.871x+0.565	0.733	x=4.37	78	39	39
0.5≤1.0	0.899	<0.001	y=0.758x+1.226	0.158	x=5.06	287	144	143
1.0≤1.5	0.883	<0.001	y=0.676x+1.640	0.147	x=5.05	169	78	91
>1.5	0.809	<0.001	y=0.623x+2.441	0.265	x=6.47	91	29	62

Table 3.8.: Comparison of **MS_F** from MCTQ and sleep log. People were grouped depending on standard deviation (StDev) of actual MS_F calculated from sleep log data. Bonferoni corrected level of significance: 0.0007.

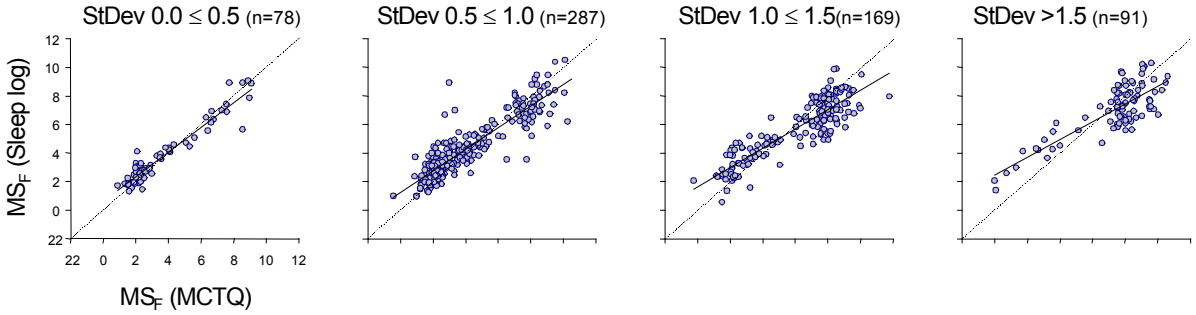


Fig.3.9.: Graphs of regressions of **$MS_{F\ (MCTQ)}$** and **$MS_{F\ (Sleep\ log)}$** presented in **Table 3.8.**

For all groups, regression of MS_F MCTQ and sleep log is highly significant but becomes slightly weaker with increasing standard deviation. Paired t-Test shows MS_F not to be significantly different between MCTQ and sleep log (**Table 3.8.**)

Early and late chronotypes are not evenly distributed in each group. Individuals with standard deviations <1.0 consist mostly of earlier types and older age. Average MS_{FSc} is significantly different between groups ($p<0.001$) except for groups with standard deviations ≤0.5 and 0.5≤1.0 (Scheffé test: $p=0.690$). Groups are also significantly different for age ($p<0.001$; groups with standard deviations ≤0.5 and 0.5≤1.0: $p=0.01$), except the two groups with standard deviations >1 (Scheffé test: $p=0.98$). Results are summarized in **Fig.3.10.**

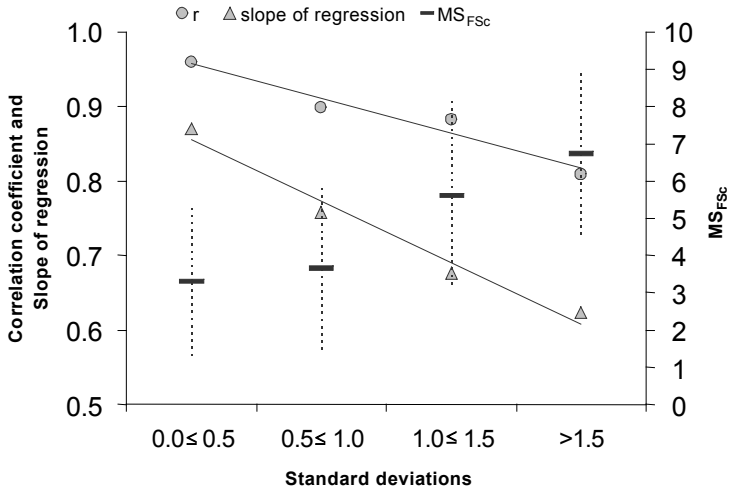


Fig.3.10.: Properties of groups presented in **Fig.3.2.8.**

Correlation coefficient r and slope of regression are shown as circles and triangles, respectively, with values on left y-axis. Average chronotype (MS_{FSc}) is indicated as bars with standard deviations (dotted lines).

	MS_{FSc}	Age
0.0≤0.5	3.29±2.06	46.4±12.9
0.5≤1.0	3.64±2.27	41.1±14.2
1.0≤1.5	5.62±2.52	30.7±11.7
>1.5	6.72±2.23	30.0±11.0
ANOVA	p<0.001	p<0.001

For descriptions see text.

Despite the fact that people with high standard deviations of sleep times on free days belong more often to the late half of the chronotype distribution (standard deviation of MS_F is significantly lower in early half, t-Test: p<0.001), there are also late types that maintain identical sleep times on free days. Still, this is rather the rule for earlier types. Three exemplary sleep logs are shown in **Fig.3.11.**

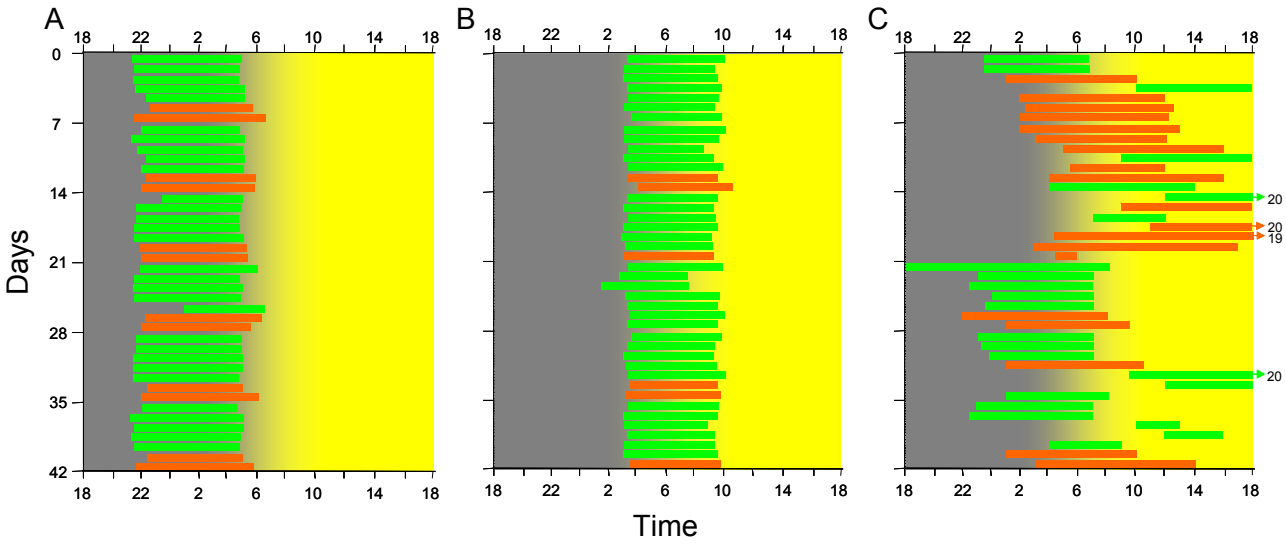


Fig.3.11.: Three sleep logs with either very low (A and B) and very high (C) standard deviations of average MS_F. A total of six weeks of documentation is shown (y-axis). Each bar represents sleep period of one day (green: work days; orange: free days). Time ranks from 18:00 to 18:00 of the next day (x-axis) and sun rise is arbitrarily set at about 6 am.

	Age	Gender	Ø MS_F (MCTQ)	Ø MS_F (sleep log)	StDev MS_W (sleep log)	StDev MS_F (sleep log)
A:	43	female	1.81	1.75	0.58	0.20
B:	47	male	6.33	6.42	0.37	0.28
C:	24	male	7.58	6.38	6.59	4.33

3.2.3.2. Differences between MS_W and MS_F

There can be big differences between sleep times on work days and sleep times on free days. It has been shown that, on average, with increasing chronotype SLD_W decreases while SLD_F is prolonged, compared to $SLD_Ø$ (Roenneberg *et al*, 2003; Valdez *et al*, 1996; see also **Fig.2.7.**). A prolonged and delayed SLD_F is accompanied by a delay of MS_F that can amount to many hours in extreme cases. On the other hand, in some cases MS_F is even earlier than MS_W. Again, four groups a created according to differences between MS_W and MS_F. The first group contains subjects with MS_F earlier than MS_W. Subjects differing not or in maximum 2 hours between MS_W and MS_F fall into the second group, the third group differs 2 to 4 hours and the fourth group differs more than 4 hours between MS_W and MS_F. A comparison of MS_F from MCTQ and sleep log for these groups is shown in **Table 3.9.** and **Fig.3.12.**

Diff	r	p (r)	Regression	p (paired t-Test)	Intercept with 1:1	n_{All}	n ♀	n ♂
<0	0.941	<0.001	y=0.852x+1.139	0.228	x=7.71	51	20	31
0<2	0.895	<0.001	y=0.759x+1.217	0.061	x=5.05	351	182	169
2<4	0.751	<0.001	y=0.746x+1.292	0.001	x=5.08	130	51	79
≥4	0.442	0.001	y=0.762x+0.909	<0.0001	x=3.82	58	24	34

Table 3.9.: Comparison of $\mathbf{MS_F}$ from MCTQ and sleep log. People were grouped for differences between MS_W and MS_F (MS_W subtracted from MS_F, left column). Bonferoni corrected level of significance: 0.0007.

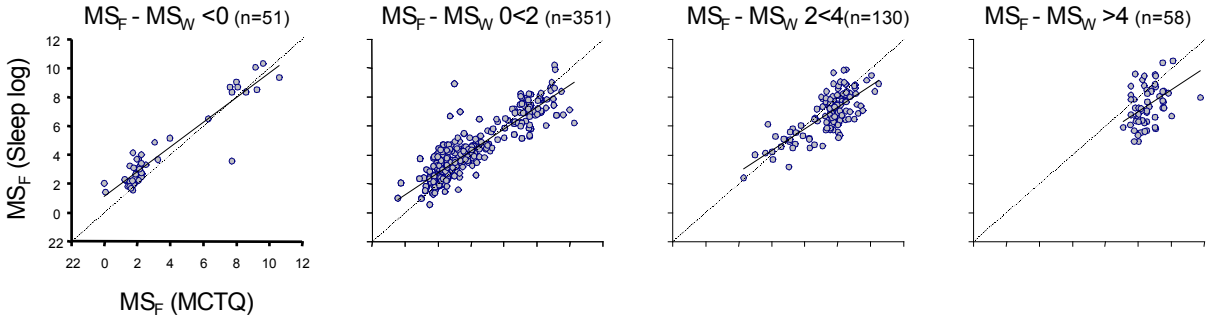


Fig.3.12.: Graphs of regressions of $\mathbf{MS_{F\ (MCTQ)}}$ and $\mathbf{MS_{F\ (Sleep\ log)}}$ presented in **Table 3.9.**

Again, chronotypes are not evenly distributed in groups. The group differing more than 4 hours between MS_W and MS_F consists exclusively of late types with MS_{FSc} >7. In general and as already seen for variances of sleep times on free days, the higher the difference the later and younger, on average, the individuals in each group. These findings are summarized in **Fig.3.13.**

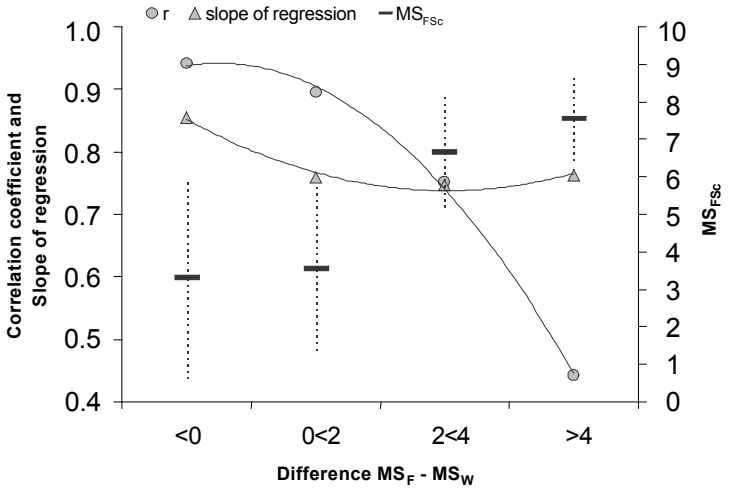


Fig.3.13.: Properties of groups presented in **Table 3.9.** and **Fig.3.12.** Correlation coefficient r and slope of regression are shown as circles and triangles, respectively, with values on left y-axis. Average chronotype (MS_{FSc}) is indicated as bars with standard deviations (dotted lines). Labels of x-axis refer to the difference between MS_W and MS_F (MS_W subtracted from MS_F).

	MS_{FSc}	Age
<0	3.30±2.69	47.9±12.2
0<2	3.56±2.27	39.0±12.7
2<4	6.64±1.49	29.1±9.4
>4	7.54±1.11	25.7±9.6
ANOVA	p<0.001	p<0.001

For details see text.

ANOVA revealed highly significant differences between groups for MS_{FSc} and Age. Sub analysis showed differences not to be significant for MS_{FSc} between *<0* and *0<2* (Scheffé: p=0.972) and significant for *2<4* and *>4* (Scheffé: p=0.55, Student-Newman-Keuls: 0.006, Tukey: 0.029). For Age, only *2<4* and *>4* are not significantly different (Scheffé: p=0.259). Correlation becomes drastically weaker with increasing difference between MS_W and MS_F whereas regression is more or less constant between *0<2*, *2<4*, and *>4*. Sleep logs exemplary for large differences between MS_W and MS_F are shown in **Fig.3.14.**

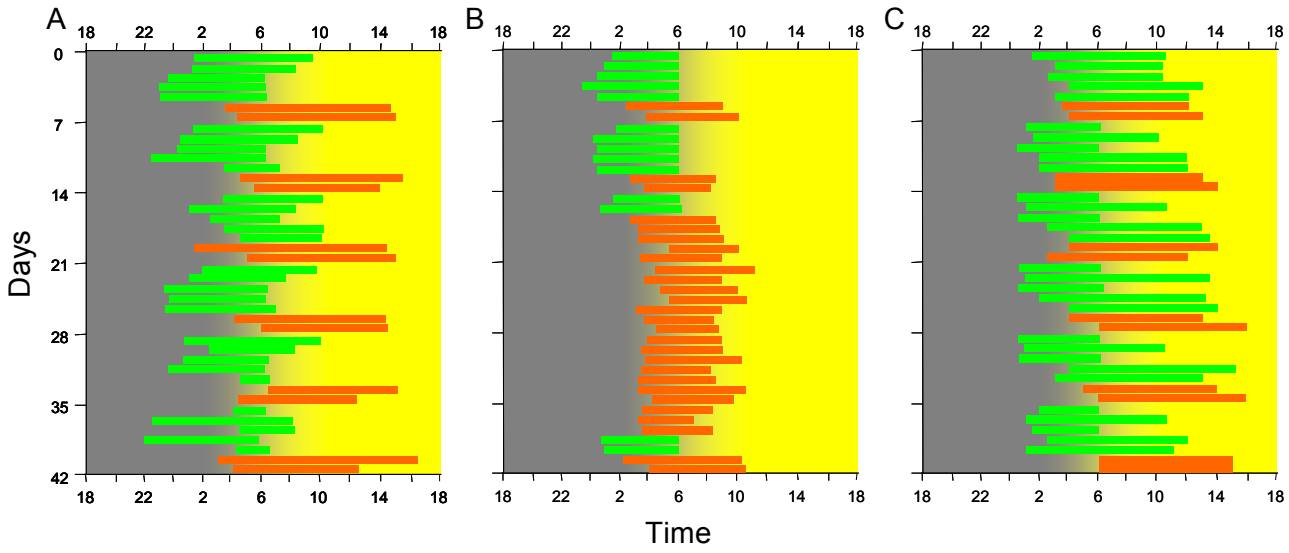


Fig.3.14.: Three sleep logs with MS_W largely different from MS_F. A total of six weeks of documentation is shown (42 days, y-axis). Each bar represents sleep period of one day (green: work days; orange: free days). Time ranks from 18:00 to 18:00 of the next day (x-axis) and sun rise is arbitrarily set at about 6 am.

	Age	Gender	Ø MS_W (sleep log)	Ø MS_F (sleep log)	StDev MS_W (sleep log)	StDev MS_F (sleep log)	Difference (MS_F) MCTQ-Sleep log
A:	20	male	4.45	9.43	1.47	0.92	0.345
B:	57	female	3.38	6.46	0.29	0.74	0.132
C:	21	female	5.91	9.06	2.01	1.19	0.893

3.3. Classification of chronotypes

3.3.1. Correlations of MCTQ variables

It has been shown by Roenneberg *et al* (2004a) that the distribution of chronotype is almost the same for short sleepers ($SLD_{Ø}$: 3-6 hours) and long sleepers ($SLD_{Ø}$: 9-12 hours). $SLD_{Ø}$, therefore, couldbe independent of MS_{FSc}. The independence of MS_{FSc} and $SLD_{Ø}$ results in a very weak correlation between both variables (r=-0.07) and a slope of the regression line close to 0 (y=0.051+7.67). However, due to the high number of the dataset used (n=11771) results are still highly significant.

In order to scrutinize if other independent characteristics of chronotype can be determined, every MCTQ variable is correlated to each other. Following variables will be used for classification of potential chronotype sub-groups: 1) $BT_{W/F}$, 2) $SO_{W/F}$, 3) $MS_{W/F}$, 4) $SE_{W/F}$, 5) $IWT_{W/F}$ 6) $FU_{W/F}$, 7) $DIP_{W/F}$, 8) $SLD_{W/F}$, 9) MS_{FSc} and 10) $SLD_{Ø}$

First, all variables were checked for linear relation; no polynomic, logarithmic, u-shaped, parabolic, s-shaped, or cubic relations could be observed. Correlation coefficients of every single combination are presented in **Table 3.10.**

	BT_W	SO_W	SE_W	IWT_W	FA_W	DIP_W	MS_W	SLD_W	BT_F	SO_F	SE_F	IWT_F	FA_F	DIP_F	MS_F	SLD_F	MS_{FSc}	$SLD_Ø$
BT_W	1.00	0.97	0.60	0.61	0.54	0.22	0.88	-0.45	0.73	0.73	0.43	0.44	0.42	0.26	0.64	-0.19	0.66	-0.42
SO_W		1.00	0.61	0.62	0.57	0.21	0.90	-0.47	0.73	0.75	0.45	0.46	0.46	0.27	0.66	-0.20	0.68	-0.44
SE_W			1.00	0.97	0.80	0.25	0.89	0.41	0.45	0.46	0.45	0.45	0.43	0.20	0.52	0.09	0.62	0.35
IWT_W				1.00	0.83	0.25	0.89	0.37	0.47	0.48	0.48	0.49	0.47	0.22	0.54	0.09	0.63	0.32
FA_W					1.00	0.24	0.76	0.24	0.46	0.47	0.51	0.53	0.57	0.25	0.56	0.14	0.60	0.24
DIP_W						1.00	0.26	0.03	0.13	0.13	0.08	0.08	0.07	0.45	0.12	-0.03	0.14	0.01
MS_W							1.00	-0.04	0.66	0.68	0.50	0.51	0.49	0.26	0.66	-0.06	0.72	-0.06
SLD_W								1.00	-0.34	-0.35	-0.01	-0.02	-0.04	-0.10	-0.19	0.32	-0.10	0.90
BT_F									1.00	0.99	0.56	0.56	0.56	0.34	0.85	-0.30	0.92	-0.39
SO_F										1.00	0.56	0.57	0.57	0.35	0.86	-0.30	0.93	-0.41
SE_F											1.00	0.99	0.80	0.42	0.91	0.62	0.77	0.28
IWT_F												1.00	0.82	0.42	0.90	0.60	0.77	0.26
FA_F													1.00	0.47	0.79	0.38	0.70	0.14
DIP_F														1.00	0.44	0.15	0.39	0.00
MS_F															1.00	0.23	0.95	-0.04
SLD_F																1.00	0.00	0.70
MS_{FSc}																	1.00	-0.07
$SLD_Ø$																		1.00

Table 3.10.: All possible correlations of MCTQ variables ($BT_{W/F}$, $SO_{W/F}$, $SE_{W/F}$, $IWT_{W/F}$, $FA_{W/F}$, $DIP_{W/F}$), $MS_{W/F}$, MS_{FSc}, $SLD_{W/F}$, and $SLD_{Ø}$. Correlation coefficients of MS_{FSc} with other variables are typed in red, correlation coefficients of DIP_W with other variables in green, correlations of DIP_F with other variables is typed in orange.

As expected from data shown by Roenneberg *et al* (2004a), MS_{FSc} shows weak correlation with $SLD_{ø}$. Additionally, very weak correlation of MS_{FSc} with DIP_W can be observed (r =0.14), a better correlation with DIP_F (r=0.39). DIP itself correlates rather weakly with other variables (DIP_W: $r \leq 0.25$; DIP_F: $r \leq 0.47$).

Taking MS_{FSc} as basis for chronotyping, further sub-grouping of chronotype could be performed using $SLD_{ø}$, which could approximately reflect individual sleep need.

3.3.2. Normalization for MS_{FSc}

To investigate for dependence, all variables mentioned above are normalized for MS_{FSc}: For every individual, the value of MS_{FSc} is subtracted from $BT_{W/F}$, $SO_{W/F}$, $MS_{W/F}$, $SE_{W/F}$, $IWT_{W/F}$, $FU_{W/F}$, $DIP_{W/F}$, and from MS_{FSc} itself. Average and variance of MS_{FSc} are thus set to 0. The higher the dependency of a variable on MS_{FSc}, the more the respective variance will decrease. Variables normalized to MS_{FSc} are shown as 24-hour-dials in **Fig.3.15.**

Both, DIP_W and DIP_F show by far the greatest standard deviation (2.36 and 2.34, respectively), indicating a relative independence of MS_{FSc}. Lowest standard deviations are apparent for MS_F (0.43), BT_F (0.58) and SO_F (0.53).

Normalization for Gender and Age groups shows different standard deviations. The early ($MS_{FSc} \leq 4.28$) and older (Age >30) group show lower standard deviations for all variables than the late (MS_{FSc} >4.28) and the younger (Age ≤30) group, respectively. Despite the differences between groups, DIP_W and DIP_F by far show the highest standard deviations for every group. Results for different groups are summarized in **Table 3.11.** and graphically shown in **Fig.3.16.**

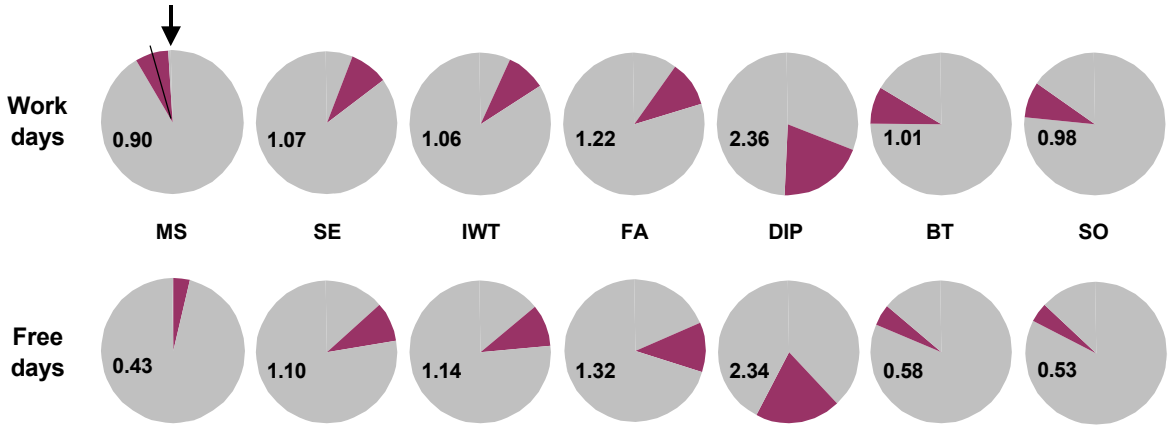


Fig.3.15.: Variation of variables after normalization to MS_{FSc}. A dial represents 24 hours,starting at MS_{FSc} set to 0 (indicated by the arrow in upper left graph). Coloured sectors span 2 standard deviations with average value of normalized variable in the middle (black line in upper left graph). For example, MS_W, on average, is earlier than MS_{FSc}, the coloured sector therefore lies counter-clockwise of MS_{FSc} set to 0. Values of respective standard deviations are shown in each graph.

		MS_W	SE_W	IWA_W	FA_W	DIP_W	BT_W	SO_W	MS_F	SE_F	IWA_F	FA_F	DIP_F	BT_F	SO_F
All	Ø	-1.11	2.50	2.75	3.58	9.66	18.99	19.28	0.38	4.39	4.63	5.83	11.41	20.12	20.36
	StDev	0.89	1.06	1.06	1.21	2.36	1.01	0.98	0.44	1.11	1.15	1.31	2.37	0.56	0.53
F	Ø	-1.09	2.59	2.85	3.74	9.68	18.91	19.22	0.37	4.45	4.69	5.94	11.44	20.04	20.29
	StDev	0.85	1.02	1.02	1.19	2.22	0.96	0.94	0.44	1.11	1.16	1.32	2.27	0.55	0.52
M	Ø	-1.12	2.40	2.63	3.42	9.64	19.08	19.35	0.39	4.34	4.57	5.71	11.38	20.21	20.43
	StDev	0.93	1.10	1.09	1.22	2.51	1.05	1.01	0.44	1.11	1.14	1.29	2.47	0.56	0.53
<30	Ø	-1.34	2.33	2.59	3.47	9.15	18.67	18.99	0.45	4.63	4.88	6.04	11.42	20.02	20.27
	StDev	0.95	1.14	1.14	1.26	2.27	1.02	1.00	0.47	1.16	1.20	1.35	2.39	0.56	0.53
>30	Ø	-0.83	2.71	2.94	3.73	10.27	19.39	19.64	0.29	4.11	4.33	5.58	11.40	20.25	20.47
	StDev	0.73	0.91	0.92	1.14	2.33	0.84	0.82	0.37	0.97	1.01	1.21	2.34	0.53	0.51
<4.28	Ø	-0.70	2.95	3.15	3.91	10.38	19.38	19.65	0.35	4.39	4.59	5.84	11.64	20.09	20.32
	StDev	0.57	0.72	0.75	1.02	2.23	0.74	0.72	0.40	1.04	1.08	1.27	2.38	0.51	0.49
>4.28	Ø	-1.63	1.92	2.23	3.17	8.74	18.49	18.81	0.41	4.41	4.69	5.81	11.12	20.17	20.42
	StDev	0.96	1.15	1.18	1.31	2.20	1.08	1.06	0.48	1.20	1.24	1.36	2.32	0.61	0.58

Table 3.11.: Averages (Ø) and standard deviations (StDev) for normalized variables for the total sub sample (All), Gender (F: Females, M: Males), Age (≤30 years, >30 years), and Chronotype groups (MS_{FSc} ≤4.28, MS_{FSc} >4.28).

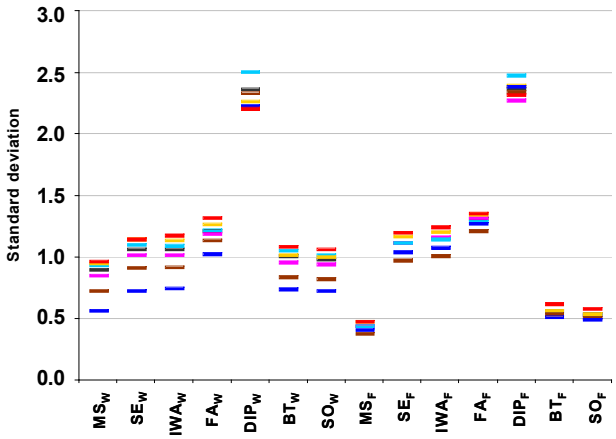


Fig.3.16.: Normalization to MS_{FSc} performed for Gender, Age, and Chronotype groups for variables shown in **Fig.3.15** and **Table 3.11.** Standard deviations of each group are shown as coloured bars.

Grey:	All
Pink:	Females
Light blue:	Males
Gold:	≤ 30 years
Brown:	>30 years
Dark blue:	MS_{FSc} ≤ 4.28
Red:	MS_{FSc} > 4.28

3.3.3. Can one determine sub-groups within the sample population of chronotypes?

Although correlating more or less highly with MS_{FSc} (all variables except for DIP_W and DIP_F), many individuals differ markedly from the calculated regression line (e.g., red ellipses in **Fig.3.17.**)

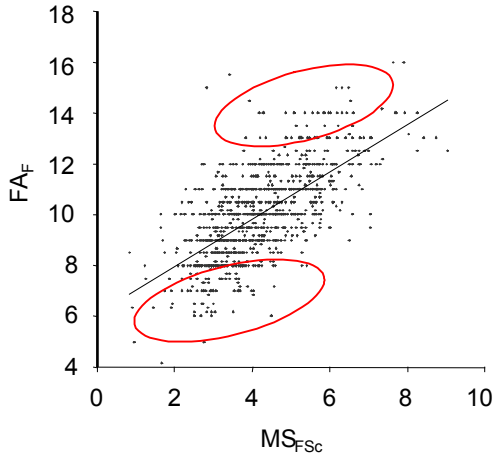


Fig.3.17.: Correlation plot of MS_{FSc} and FA_F (r=0.70, regression: y=0.99+5.85) examplary for potential sub-grouping. The regression line minimizes the squared differences between predicted y-values and actual y-values and maximizes the precision in predicting the dependent variable (FA_F) using the independent variable (MS_{FSc}). Nevertheless, many individuals differ greatly from the regression line and could represent another sub-group independent of MS_{FSc}. Red ellipses show hypothetical sub-groups.

To scrutenize sub-groups within correlating variables, the following strategy is applied:

1. MS_{FSc} is correlated with other variables and regressions are determined
2. The resulting regression line is used to predict values (y) of each variable from MS_{FSc} values
3. Actual y-values are subtracted from predicted y-values
4. Differences between actual and predicted values are ranked

If there was any grouping, a non random distribution of deviations from regression line (residuals), resulting from a stepwise increase (at least partially) of ranked values, has to be expected. In case of no hidden groups, an even increase of ranked values and a random, or at least unimodal and more or less bell shaped, distribution should be observed. Both, hypothetical results are examplarily shown in **Fig.3.18.**

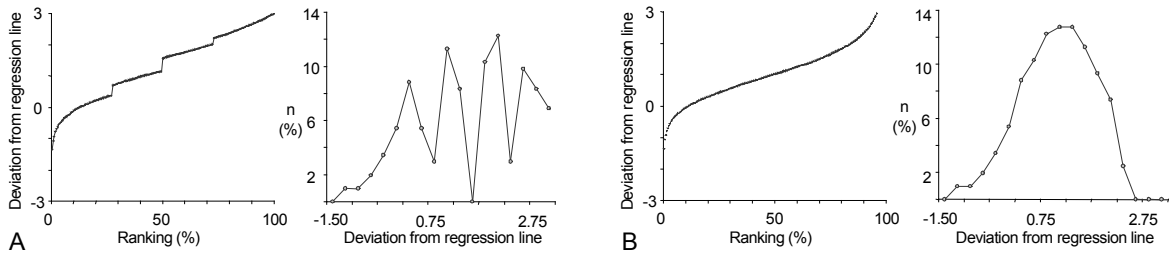


Fig.3.18.: Hypothetical outcomes of the procedure described in text above (1.-4.).

A) The even increase of deviations from regression line is interrupted by rapid increases of values, enclosing potential distinct groups (left graph). Plotting the frequencies of deviations (0.25 hour bins) results in a multimodal distribution (right graph).

B) Analogous to a), the case of a random distribution of deviations is shown as even increase of values (left graph) and a unimodal, bell shaped distribution (right graph).

MS_{FSc}, as the basis of chronotyping, was correlated with all other variables provided by the MCTQ. Exept for DIP_W and DIP_F, and the procedure described above was applied. Results are shown in **Fig.3.19.**

Neither on work days nor on free days, a clear grouping, defined by rapid increase of values of deviation from regression line (residuals), can be observed. This suggests that variables, except for DIP_W and DIP_F, are highly dependent on MS_{FSc} and do not contribute substantially to the explanation of total variance of chronotype assessed with the MCTQ.

Applying the procedure separately to Gender and Age (≤30 and >30) groups gives results comparable to those from the total sample.

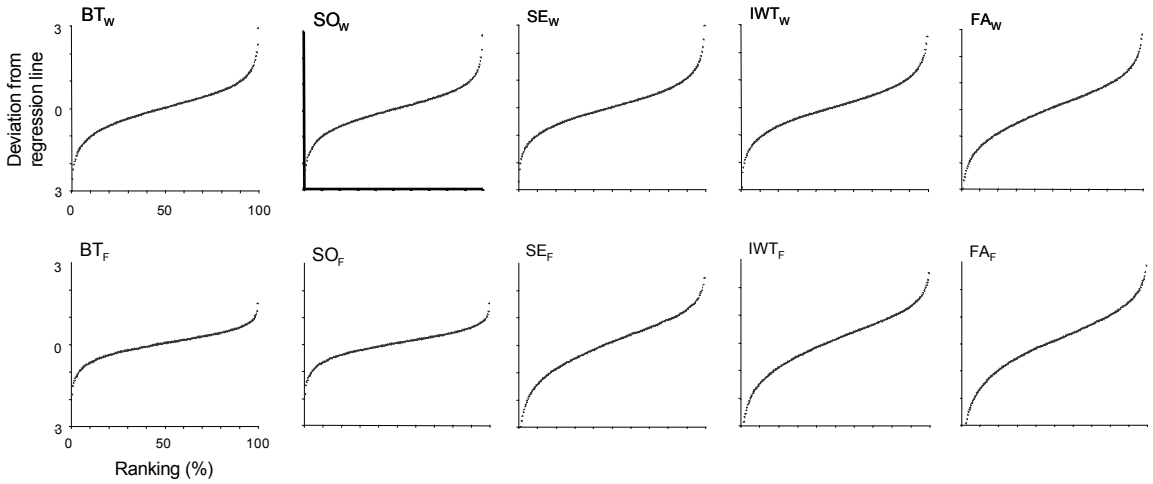


Fig.3.19.: Ranking of deviations from regression line of each correlation of MS_{FSc} with $BT_{W/F}$, $SO_{W/F}$, $SE_{W/F}$, $IWT_{W/F}$, and $FA_{W/F}$. The total sample (n=11771) is shown as 100%.

3.3.4. Confirmatory Factor Analysis

The results of chapters 3.3.1. and 3.3.2. suggested an independency of MS_{FSc} and $SLD_{Ø}$ and $DIP_{W/F}$. That gives rise to the presumption that, besides MS_{FSc}, two independent variables are apparent that could be used for further defining chronotype. However, an independency of $SLD_{Ø}$ and $DIP_{W/F}$ has not been proven so far. A common method to reveal independent structures underlying a set of variables is Factor Analysis. Based on a correlation matrix, Factor Analysis assigns variables to factors, depending on the strength of correlation, and succesively explains maximum variance with a reduced number of new variables (factors). An introduction to Factor Analysis is available in Zöfel (2001), for more detailed explanations see Backhaus *et al* (2003), Bortz (1999), and Field (2002).

3.3.4.1. Conditions of confirmatory Factor Analysis (Principle Component Analysis)

Previous results of chapter 3 suggest that mid-sleep is sufficient to determine phase of sleep and activity; in addition, other variables ($BT_{W/F}$, $IWT_{W/F}$, $FA_{W/F}$) cannot be divided further into sub-groups.

Principle Component Analysis (PCA), a special case of Factor Analysis, can be either used as an exploratory or confirmatory method, depending on the question (Backhaus *et al*, 2003). In the first case, the aim is the identification of underlying dimensions of a set of variables in order to create hypotheses. In the latter case, and as it is used here, a prior hypothesis about underlying structures is tested. Although a PCA is performed, the common term "Factor Analysis" will be used and applied with following conditions:

- Variables: $BT_{W/F}$, $SO_{W/F}$, $SE_{W/F}$, $IWT_{W/F}$, $FA_{W/F}$, $DIP_{W/F}$, $MS_{W/F}$, and $SLD_{W/F}$. Furthermore, MS_{FSc} and $SLD_{Ø}$ are included.
- Separate analyses for Gender and Age groups are performed (Females: n=6124, Males: n=5647, Age≤30: n=6471, Age >30: n=5300)
- Factors with eigenvalues > 1 are extracted (Kaiser's criterion).
- No rotation is performed in order to keep up comparability of separate analyses (Bortz 1999, p. 537)

3.3.4.2. Testing assumptions of sampling adequacy

a) Multicollinearity

The correlation matrices of each group show high correlation coefficients for variables for which a high dependency on MS_{FSc} has been shown. No significant correlations are apparent between variables for which independency is assumed (MS_{FSc} and dependents versus $SLD_{Ø}$ or $DIP_{W/F}$). Determinants of correlation matrices are much smaller than recommended (1.59^{-36} for Females to 8.12^{-38} for Age ≤30 years, recommended: $>10^{-5}$). These outcomes could have been predicted from former results when including all variables that highly correlate with MS_{FSc}, and improve when eliminating highly correlating variables. Nevertheless, it is of interest to include all variables in one approach and violation of assumption of no multicollinearity is accepted in this case.

b) Anti-Image-Matrix

The multiple Kaiser-Meyer-Olkin measure of sampling adequacy results in values >0.8 which is "meritorious" (for classification of K-M-O values see **Table 3.12.**). Individual results show values slightly below the critical value of 0.5 only for SLD_F. Removing SLD_F from the analysis improves results with no value under 0.5 and a determinant of correlation matrix of about 10^{-30}, although the latter improvement is neglectable. All results are summarized in **Table 3.12.**

	All	Females	Males	Age <30	Age >30
BT_W	0.918	0.914	0.921	0.925	0.917
SO_W	0.852	0.842	0.858	0.842	0.869
SE_W	0.823	0.808	0.836	0.798	0.839
IWT_W	0.958	0.960	0.955	0.958	0.956
FA_W	0.966	0.963	0.971	0.960	0.970
DIP_W	0.686	0.703	0.665	0.753	0.697
MS_W	0.938	0.935	0.940	0.941	0.935
SLD_W	0.630	0.628	0.618	0.631	0.659
BT_F	0.926	0.922	0.928	0.929	0.921
SO_F	0.799	0.787	0.807	0.792	0.807
SE_F	0.773	0.755	0.788	0.753	0.780
IWT_F	0.962	0.962	0.960	0.959	0.960
FA_F	0.950	0.941	0.958	0.939	0.957
DIP_F	0.847	0.842	0.850	0.848	0.822
MS_F	0.971	0.971	0.969	0.973	0.966
SLD_F	**0.499**	0.512	**0.487**	**0.483**	**0.495**
$SLD_Ø$	0.883	0.889	0.870	0.893	0.875
MS_{FSc}	0.984	0.980	0.985	0.951	0.978
Overall	0.884	0.877	0.890	0.875	0.888

Table 3.12.: Kaiser-Meyer-Olkin Measures of sampling adequacy (MSA). The overall measure is "meritorious" for every group, only SLD_F is below 0.5, except for Females. MSA is classified as follows:

MSA ≥ 0.9: marvelous
MSA ≥ 0.8: meritorious
MSA ≥ 0.7: middling
MSA ≥ 0.6: mediocre
MSA ≥ 0.5: miserable
MSA < 0.5: unacceptable

(Backhaus *et al*, 2003, p. 276)

c) Bartlett's test of spericity

For Factor Analysis, it is important that there are some relationships between variables. If there were no relationships between variables then all correlation coefficients would be 0 and the correlation matrix is an identy matrix. The null hypothesis that the correlation matrix is an identity matrix is tested with Bartlett's measure. In this case, the null hypothesis can be rejected with very high significance ($p<0.001$) for each single group.

3.3.4.3. Results of confirmatory Factor Analysis

a) Factor extraction

For every group, four factors with eigenvalues > 1 were extracted. A fifth factor was extracted for Age≤30 (eigenvalue = 1.005). Factor solutions for all groups are presented in **Table 3.13.**, the respective scree plots are shown in **Fig. 3.20.**

		All	Females	Males	Age≤30	Age>30
Factor 1	Eigenvalue	9.91	9.51	10.27	9.57	10.25
	% of variance	52.16	50.05	54.07	50.38	53.97
Factor 2	Eigenvalue	3.53	3.62	3.38	3.38	3.60
	% of variance	18.57	19.07	17.80	17.78	18.95
Factor 3	Eigenvalue	2.18	2.28	2.13	2.59	1.81
	% of variance	11.49	12.00	11.20	13.65	9.52
Factor 4	Eigenvalue	1.26	1.25	1.27	1.21	1.35
	% of variance	6.61	6.56	6.68	6.34	7.09
Factor 5	Eigenvalue				1.01	
	% of variance				5.29	

Table 3.13.: Factors with eigenvalues >1 were extracted using Kaiser's criterion. Only for Age ≤ 30, 5 factors were extracted. Eigenvalues and the respective amount of variance explained by each factor are shown. For details see text.

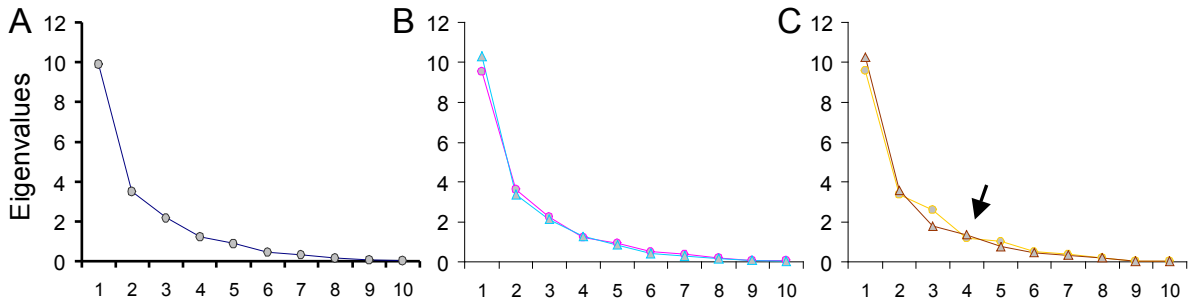


Fig.3.20.: Scree plots of eigenvalues. A: total sample, B: Females (pink circles), Males (blue triangles), C: Age ≤ 30 (golden circles), Age>30 (brown triangles). Only 3 factors are obtained for Age ≤ 30 using the scree plot method (black arrow indicates elbow). For all other groups, no clear elbow can be determined.

For factor 5 of Age≤30, no substantial loading for any variable can be observed (≤ 0.391; for substantial factor loadings see text below). Taking the scree plot solution into account and the extraction solution of the other groups, the fifth factor of Age≤30 will be discarded and not used for further analyses.

b) Communalities

In Factor Analysis, as many factors are extracted as there are variables and all factors explain all variance within a set of variables. Many factors, however, do not contribute substantially to the explanation of variance and are thus discarded (here: all factors with eigenvalues <1). Therefore, not all variance can be explained by the retained factors. The communalities represent the amount of variance in each variable explained by the retained factors. **Table 3.13.** shows the communalities for all groups and each variable. For Age≤30, communalities of the four factor solution are shown.

	All	Females	Males	Age < 30	Age > 30
BT_W	0.846	0.826	0.858	0.832	0.856
SO_W	0.880	0.862	0.892	0.863	0.891
SE_W	0.980	0.978	0.983	0.978	0.978
IWT_W	0.968	0.963	0.973	0.966	0.967
FA_W	0.762	0.716	0.805	0.742	0.769
DIP_W	0.826	0.804	0.838	0.781	0.829
MS_W	0.945	0.936	0.953	0.938	0.943
SLD_W	0.858	0.854	0.860	0.838	0.887
BT_F	0.890	0.880	0.897	0.891	0.901
SO_F	0.911	0.906	0.914	0.916	0.921
SE_F	0.973	0.969	0.975	0.968	0.979
IWT_F	0.971	0.968	0.974	0.967	0.976
FA_F	0.754	0.716	0.790	0.741	**0.690**
DIP_F	0.761	0.743	0.774	0.725	0.790
MS_F	0.980	0.978	0.982	0.981	0.977
SLD_F	0.835	0.844	0.825	0.836	0.877
$SLD_Ø$	0.981	0.979	**0.984**	0.977	0.980
MS_{FSc}	0.922	0.914	0.930	0.916	0.923

Table 3.13.: Communalities of variables for different groups. High values are obtained for all groups indicating a large amount of variance explained by the extracted factors. Highest and lowest values are shown as bold numbers.

c) Factor loadings

All results, presented so far, tested the adequacy of the data matrix used and the variance that can be explained by the extracted factors. Almost 90% of the total variance can be explained by the four factors. There are almost no differences in explained variance between Gender and Age groups (All groups: 88.8%, Females: 87.7%, Males: 89.8%, Age≤30: 88.2%, Age >30: 89.5%).

In Factor Analysis, all factors are assumed to be mathematically independent. Factor loadings represent the Pearson's correlation between a variable and a factor. If 1) a variable loads highly on the first factor and low on the second factor and 2) another variable loads highly on the second factor and low on the first factor, then these two variables are highly independent of each other (they correlate weakly). Therefore, MS_{FSc}, $SLD_{Ø}$, and $DIP_{W/F}$ should highly load on different factors in case they are really independent of each other. The loadings of all variables on the four extracted factors are shown for the total sample in **Table 3.14.**

All groups	% of explained variance			
	52.2	18.6	11.5	6.6
BT_W	0.81	-0.39	0.21	0.01
SO_W	0.83	-0.39	0.20	0.00
SE_W	0.74	0.33	0.57	-0.06
IWT_W	0.76	0.32	0.53	-0.05
FA_W	0.74	0.29	0.37	-0.01
DIP_W	0.24	0.02	0.24	0.84
MS_W	0.87	-0.04	0.43	-0.03
SLD_W	-0.13	0.82	0.40	-0.07
BT_F	0.85	-0.38	-0.09	-0.08
SO_F	0.87	-0.38	-0.09	-0.08
SE_F	0.79	0.37	-0.46	-0.04
IWT_F	0.80	0.36	-0.45	-0.04
FA_F	0.75	0.25	-0.35	0.01
DIP_F	0.45	0.07	-0.25	0.70
MS_F	0.93	0.03	-0.33	-0.07
SLD_F	0.09	0.79	-0.44	0.03
$SLD_Ø$	-0.06	0.98	0.10	-0.04
MS_{FSc}	0.94	-0.01	-0.17	-0.09

Table 3.14.: Factor loadings of the total sample. The highest loadings for each variable are shown in red, all additional loadings >0.5 are shown in blue. Results are separated for work day values, free day values, and average values. Factors are sorted from left to right according to the amount of variance explained:

Factor 1: 52.2%

Factor 2: 18.6%

Factor 3: 11.5%

Factor 4: 6.6%

There are many recommendations in statistical literature concerning factor loadings. The interpretability of factors depends on factor loadings and sample size (Bortz, 1999, p.534/535). Due to the high number of individuals (n=11,771) there is no concern about interpretability of factors. As a rule of thumb, loadings >0.4 will be interpreted for the respective factor (Field, 2002, p.440), values >0.5 will be assessed as high loadings (Backhaus *et al*, 2003, p.299).

According to these guidelines, four factors can be determined:

Factor 1 (F1): High loadings exist for variables which have shown to be highly correlated with MS_{FSc} (see chapter 3.3.2. and 3.3.3.). DIP_F has an interpretable loading on factor one (0.45).

Factor 2 (F2): On the second factor, SLD_W, SLD_F, and $SLD_Ø$ show high loadings. $BT_{W/F}$, $SO_{W/F}$, $SE_{W/F}$, and $IWT_{W/F}$ have loadings slighly below 0.4.

Factor 3 (F3): High loadings on the third factor are apparent only for SE_W and IWT_W.

Factor 4 (F4): On the fourth factor, only DIP_W and DIP_F show high loadings, all other variables are below 0.1.

d) Interpretation of factors (F1, F2, F4)

As mentioned above, high loadings on one factor and (relatively) low loadings on the other factors, respectively for every variable represents independence of each other. The results presented in **Table 3.14.** are graphically shown in **Fig.3.21.**

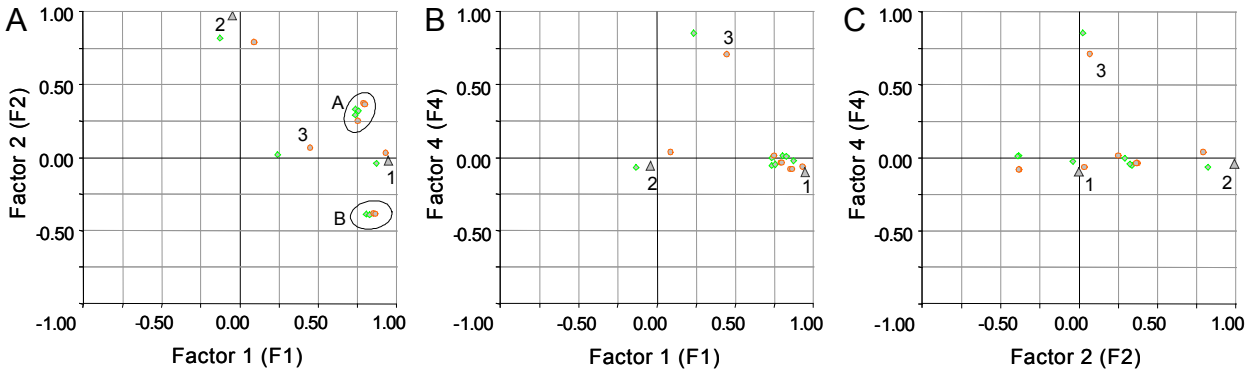


Fig.3.21.: Graphical representation of the factor loadings given in **Table 3.14.** Respective loadings of Factors 1, 2, and 4 are two-dimensionally plotted. A: Factor 1+2, B: Factor 1+4, C: Factor 2+4. Work days are represented as green diamonds, free days as orange circles. Corrected variables are shown as grey triangles and labeled with numbers: MS_{FSc} (1), $SLD_{Ø}$ (2). For example, DIP_F (3) has a loading value of 0.45 on the horizontal axis of graph B (Factor 1) and a loading value of 0.07 on the vertical axis (Factor 2).

MS_{FSc} loads highly on F1 (0.94) and practically not on F2 (-0.01) and F4 (-0.09). $SLD_{Ø}$ shows a very high loading value on F2 (0.98) and almost no loading on the other factors (F1: -0.06, F3: 0.10, F4: -0.04). SLD_W and SLD_F have lower, but still high, loadings on F2 (0.82 and 0.79, respectively). Interpretable loadings are apparent on F3 (0.40 and –0.44, respectively). DIP_W and DIP_F have high loadings on F4 (0.84 and 0.70, respectively), DIP_F also loads interpretably on F1 (0.45).

Factor Analysis (PCA) issued to find structures underlying a set of variables and to create a generic term for each structure determined. Factor 1 could be named *'Chronotype'* because MS_W, MS_F, and MS_{FSc} all have loadings >0.87 on that factor. Also, $BT_{W/F}$, $SO_{W/F}$, $SE_{W/F}$, $IWT_{W/F}$ and $FA_{W/F}$ load highly on F1 (>0.74). All these variables have been shown to highly correlate with MS_{FSc} (chapter 3.3.1.-3.3.3.). Nevertheless, these variables also load almost interpretably on F2 (-0.39 < 0.37). This is obvious because SLD is calculated by subtracting SO from SE. The opposite sign of loadings for $BT_{W/F}$ and $SO_{W/F}$ (negative, "B" in left graph of **Fig.3.21.**) and $SE_{W/F}$, $IWT_{W/F}$ and $FA_{W/F}$ (positive, "A" in left graph of **Fig.3.21.**) are logical. Lower ("earlier") values of $BT_{W/F}$ and $SO_{W/F}$ as well as higher ("later") values of $SE_{W/F}$, $IWT_{W/F}$ and $FA_{W/F}$ both are associated with higher ("longer") values for SLD_W and SLD_F, respectively.

Because of high loadings of SLD_W, SLD_F, and $SLD_Ø$, F2 could be named *'Duration (of sleep)'*. Only DIP_W and DIP_F load highly on F4 (0.84 and 0.70, respectively); no loadings of other variables are worth to be mentioned (-0.09 < 0.03). DIP_F additionally loads interpretably on F1 (0.45) while DIP_W loads weakly on F1 (0.24).

These results confirm the hypothesis derived in previous chapters. Furthermore, Factor Analysis allows to assess the contribution of every factor to the explanation of the total variance within a given set of variables. *'Chronotype'* is most dominant with about 50% explanation of total variance and MS_{FSc} is highly representative for *'Chronotype'*. *'Duration'* explains 18.6% variance and can be almost perfectly represented by $SLD_Ø$. Only 6.6% of total variance can be explained by F4. The different loadings of DIP_W and DIP_F on F4 might depend on individual social and working habits. Thus, F4 could also be labeled as *'Social conventions'*. On free days, schedules seem to be not so strict as on work days, due to a lower loading value on work days (0.24) than on free days (0.45).

e) Interpretation of factors (F3)

None of the variables has its highest loading on F3. Nevertheless, SE_W and IWT_W load highly on F3 (0.57 and 0.53, respectively). Interpretable loading values are reached by MS_W (0.43), SLD_W (0.40), SE_F (-0.46), IWT_F (-0.45), and SLD_F (-0.44). The dominant variable on F3 is SE_W (among with highly correlating IWT_W).

For the majority of chronotypes, SE_W limits sleep on work days, and thus functions as a social clock besides the biological clock and the solar clock (Roenneberg *et al*, 2003a). Interpretable loadings of other variables on F3 indicate an influence of SE_W on those variables. Indeed, positive values for work day variables and negative values for free day variables reflect results shown before from Roenneberg *et al* (2003a): decreasing ("earlier") values of SE_W lead to decreasing values of MS_W ("advanced") and SLD_W ("shortened"). The opposite can be observed for free days. With decreasing ("earlier") values of SE_W, values for SLD_F increase ("prolong"). In the same way, values for SE_F, IWT_F, FA_F, and MS_F increase ("delay").

BT_W and SO_W are less influenced (loadings: 0.21 and 0.20, respectively), while almost no influence can be seen for BT_F and SO_F (loadings: -0.09 for both). This could be interpreted as follows: the social clock (e.g. work times) is not able to advance the biological clock, SO_W cannot occur early enough to compensate for early rise times. This is compensated for on free days by sleeping in (SE_F: -0.46) and not by advancing sleep onset (SO_F: -0.09). In summary, Factor 3 could be named *'Social jet-lag'*.

f) Differences between Gender and Age groups

Factor solutions found for the total sample (n=11,771) also apply to each group (Females, Males, Age≤30, and Age >30). F1 ('Chronotype') is the dominant factor for all groups with slightly higher explained variance for Males and Age >30 (54.1% and 54.0%, respectively; Females: 50.1%, Age≤30: 50.4%). F2 ('Sleep duration') explains more variance for Females and Age>30 (19.1% and 18.9%, respectively; Males: 17.8%, Age≤30: 17.8%). Small differences between Females and Males can be seen for F3 ('Social jet-lag) with 12.0% and 11.2%, respectively. In contrast to this rather small difference, F3 explains 13.6% of variance for Age≤30 but only 9.5% for Age>30. Factor loadings of all groups are presented in **Table 3.15.**

Females	% of explained variance			
	50.1	19.1	12.0	6.6
BT_W	0.79	-0.39	0.20	0.03
SO_W	0.81	-0.41	0.19	0.03
SE_W	0.71	0.33	0.60	-0.06
IWT_W	0.74	0.32	0.56	-0.04
FA_W	0.71	0.28	0.36	0.00
DIP_W	0.25	0.03	0.22	0.83
MS_W	0.86	-0.04	0.44	-0.01
SLD_W	-0.12	0.80	0.43	-0.09
BT_F	0.84	-0.40	-0.09	-0.10
SO_F	0.85	-0.41	-0.08	-0.10
SE_F	0.76	0.41	-0.47	-0.04
IWT_F	0.77	0.40	-0.46	-0.04
FA_F	0.72	0.27	-0.35	0.02
DIP_F	0.45	0.08	-0.24	0.69
MS_F	0.92	0.05	-0.34	-0.08
SLD_F	0.07	0.81	-0.43	0.04
$SLD_ø$	-0.06	0.98	0.12	-0.05
MS_{FSc}	0.93	0.00	-0.18	-0.11

Males	% of explained variance			
	54.1	17.8	11.2	6.7
BT_W	0.81	-0.38	0.23	0.00
SO_W	0.84	-0.38	0.22	-0.01
SE_W	0.76	0.32	0.54	-0.06
IWT_W	0.79	0.30	0.51	-0.06
FA_W	0.77	0.27	0.38	-0.01
DIP_W	0.22	0.03	0.26	0.85
MS_W	0.88	-0.03	0.42	-0.04
SLD_W	-0.11	0.84	0.38	-0.06
BT_F	0.86	-0.37	-0.10	-0.07
SO_F	0.87	-0.37	-0.09	-0.07
SE_F	0.82	0.33	-0.45	-0.04
IWT_F	0.83	0.32	-0.44	-0.04
FA_F	0.79	0.21	-0.36	0.01
DIP_F	0.45	0.06	-0.25	0.71
MS_F	0.94	0.02	-0.32	-0.06
SLD_F	0.13	0.78	-0.45	0.03
$SLD_ø$	-0.02	0.99	0.08	-0.04
MS_{FSc}	0.94	-0.02	-0.18	-0.08

Age < 30	% of explained variance			
	50.4	17.8	13.6	6.3
BT_W	0.82	-0.30	0.26	0.05
SO_W	0.84	-0.31	0.25	0.05
SE_W	0.67	0.40	0.60	-0.06
IWT_W	0.70	0.39	0.57	-0.04
FA_W	0.69	0.34	0.38	0.02
DIP_W	0.27	0.11	0.24	0.80
MS_W	0.85	0.04	0.47	0.00
SLD_W	-0.22	0.80	0.37	-0.13
BT_F	0.86	-0.38	-0.06	-0.12
SO_F	0.87	-0.38	-0.05	-0.11
SE_F	0.75	0.36	-0.52	-0.05
IWT_F	0.76	0.35	-0.51	-0.04
FA_F	0.71	0.23	-0.42	0.03
DIP_F	0.43	0.05	-0.29	0.68
MS_F	0.92	0.02	-0.35	-0.09
SLD_F	0.00	0.75	-0.52	0.06
$SLD_ø$	-0.17	0.97	0.04	-0.07
MS_{FSc}	0.93	-0.02	-0.16	-0.13

Age > 30	% of explained variance			
	54.0	18.9	9.5	7.1
BT_W	0.84	-0.35	0.15	-0.01
SO_W	0.86	-0.36	0.15	-0.01
SE_W	0.76	0.38	0.51	-0.04
IWT_W	0.78	0.37	0.47	-0.04
FA_W	0.74	0.33	0.32	-0.02
DIP_W	0.26	0.06	0.13	0.86
MS_W	0.90	0.00	0.36	-0.03
SLD_W	-0.16	0.84	0.38	-0.03
BT_F	0.86	-0.40	-0.03	-0.05
SO_F	0.87	-0.41	-0.02	-0.06
SE_F	0.80	0.36	-0.46	-0.07
IWT_F	0.80	0.35	-0.45	-0.07
FA_F	0.75	0.23	-0.28	-0.01
DIP_F	0.42	0.07	-0.21	0.75
MS_F	0.94	0.00	-0.29	-0.07
SLD_F	0.05	0.80	-0.49	-0.02
$SLD_ø$	-0.10	0.98	0.08	-0.03
MS_{FSc}	0.95	-0.02	-0.14	-0.08

Table 3.15.: Factor loadings for Gender and Age groups. The highest loadings for each variable are shown in red, all additional loadings >0.5 are shown in blue. Results are separated for work day values, free day values, and average values. Factors are sorted from left to right according to the amount of variance explained.
Although slightly different in variance explained and in factor loadings, the basic structure of the factor solutions is highly consistent. For details see text.

The most interesting differences can be observed between Age groups for F3. Age≤30 seems to be more affected by social influences than Age >30. A more established and regular lifestyle and, in many cases, more stable employment could also reduce the impact of social influence on Age group > 30. However, these differences are also apparent between Females and Males (Roenneberg *et al*, 2004b; **Fig.3.3.1.**). In spite of these differences, the general structure of factors seems to be highly consistent between Females and Males and stable throughout all ages.

3.3.4.4. Conclusions

About 70% of the total variance within the set of variables used by the MCTQ can be explained by two factors: 'Chronotype' (Factor 1) and 'Duration' (Factor 2; All: 70.7%, Females: 69,1%, Males: 71,9%, Age≤30: 68,2%, Age>30: 72,9%). Additionally, between 9.5% and 13.6% can be explained by Factor 3 named 'Social jet-lag. This factor is dominated by SLD_W which has been shown to truncate sleep on work days of a wide range of chronotypes (Roenneberg *et al*, 2003a; Valdez *et al*, 1996). A fourth factor, assigned to DIP_W and DIP_F, explaines another 6.3 - 7.1% of total variance. An association with mealtimes and other potential influences requires further investigation. Preliminary results (Appendix 1, Fig.2) indicate that for the majority of people, DIP is linked with food intake. Therefore, one should be careful when using DIP for the classification of chronotype.

MS_{FSc} and $SLD_{Ø}$ have very low loadings on F3 (-0.17 and 0.10, respectively). Both are variables corrected for differences between sleep times on work days and on free days. A neglectable loading on F3 proofs corrections for sleep dept on work days to be sensible. Additionally, both variables don't show meaningful loadings on other factors. Communalities (see 3.3.4.3b) do not change markedly when extracting only two factors, no big loss of information has to be expected when using only MS_{FSc} and $SLD_{Ø}$. For this, the assessment of SO_W, SE_W, SO_F, and SE_F is sufficient.

3.4. Biological and social factors associated with chronotype

3.4.1. Defining biological and social factors

The results presented in chapter 3.3. suggest that the MCTQ is capable to determine two independent and convincing variables, MS_{FSc} and $SLD_{Ø}$. Furthermore, the MCTQ contains questions about additional biological as well as social factors (not to be confused with the term "factor" used in chapter 3.3.4.). Among these factors, that potentionally could be associated with both MS_{FSc} and $SLD_{Ø}$, are:

Biological: 1. **AGE**, 2. **GENDER**, 3. **BMI** (Body mass index)

Social: 4. **ØDOLE** (Average daily outside light exposure), 5. **PHOTO** (Photoperiod, Time of the year), 6. **LAT** (Latitude), and 7. **POR** (Place of residence).

The association of biological and social factors with MS_{FSc} and $SLD_{Ø}$ is analysed with Multiple Regression which is an extension of Regression analysis in the way that two or more independent variables (here: biological and social factors) are used to predict a dependent variable (here: either MS_{FSc} or $SLD_{Ø}$). The capability of a variable to predict (explain a certain amount of variance of) another variable reflects the strength of association between both variables; e.g., knowing the shoe size allows a relatively good assessment of body height. Independent variabels can be either quantitative or dichotomous (binary), whereas the dependent variable has to be quantitative. Some of the independent variables (factors) used here do not fulfill the assumptions and have to be transformed. Treatment of variables and a general description are presented in detail:

1. No transformation was necessary for **AGE** (range: 10 to 90 years)
2. **GENDER** is coded: 0 for Males and 1 for Females
3. No transformation was necessary for **BMI** (range: 15 to 50 kg/m^2)
4. **ØDOLE** is calculated as weekly outside light exposure (5 times daily outside light on work days plus 2 times daily outside light on free days divided by 7) and used as quantitative variable (range: 0 to 16 hours)
5. **PHOTO** is dichotomized: October to March: 0 , April to September: 1
6. **LAT** is determined using German postal codes and divided at about 51 degrees N. The Northern part (up to 55 degrees 30' N) is coded as 0 , the Southern part (down to 46 degrees 30' N, including data from Austria and Switzerland) is coded as 1.
7. **POR** refers to a more urban or more rural social environment, arbitrarily separated by the number of inhabitants being larger or smaller than 100,000 (which is comparable with the size of Trier or Erlangen). A list of the German, Austrian, and Swiss cities with more than 100,000 residents is shown in Appendix 4.

3.4.2. Association of chronotype with biological and social factors factors

3.4.2.1. Multiple regression model

Multiple regression was performed separately for MS_{FSc} and $SLD_{Ø}$, the two major independent variables (3.3.). An association is represented by the capability of independent variables (see 3.4.1.) to predict the dependent variables (MS_{FSc} and $SLD_{Ø}$). Furthermore, the amount of variance of the dependent variable(s) that can be explained by the independent variables is estimated. Following the recommendations of Field (2002), independent variables will be labeled as 'predictors' and dependent variables as 'outcomes'. It should be noted that no causal relationship can be derived from results.

In Multiple Regression analysis, there are several ways to determine the association of predictors and the outcome. In the stepwise method, the predictor that shows the highest simple correlation with the outcome is chosen first and this predictor explains a certain variance of the outcome. Then, the predictor is chosen that shows the highest partial correlation (controlled for the first predictor) with the outcome and this predictor explains a certain part of the remaining variance of the outcome, and so on. The order of predictors is based on purely mathematical criteria. Only if a predictor significantly improves the the ability of the model to predict the outcome, it is retained in the model ('included'). If there is no significant contribution to the model, a predictor is removed ('excluded'). There is also the possibility to include predictors in a hierarchical (blockwise) manner, e.g. first predictors A and B are tested for their ability to improve the model, then predictors C and D. The order of predictors depends not on mathematical criteria but should be based on past research (Field, 2002). Within each block, however, the stepwise method can be applied. Because cases with missing values are removed from Multiple regression, the number of cases can differ between models.

Here, the predictors were hierarchically included in the regression model, based on past research. Within each block, predictors are included in a stepwise manner to assess the contribution of each variable to the outcome of the model. AGE and GENDER are obviously causal in their influences on chronotype and are entered into the first block. This, however, can't be concluded for BMI. Therefore and because only little is known about the association of BMI and chronotype, BMI is not included in the first block amongst AGE and GENDER. The social factors ØDOLE, PHOTO, and LAT all concern the availability of light throughout the day and the year. These factors are thus entered together into the second block. Finally, BMI and POR are entered into the third block.

3.4.2.2. Testing accuracy of regression model

a) General assumptions

Several crucial assumptions have to be checked before interpreting the outcome of the regression model. F-statistics show that all included variables improve the model with high significance, except BMI for MS_{FSc}, which shows a relatively low change in F compared to all other variables for both MS_{FSc} and $SLD_{Ø}$. Although BMI is a significant predictor for MS_{FSc}, with regard to F-values of other variables, the influence of BMI on MS_{FSc} could be doubtful.

Adjusted values of r^2 (corr. r^2 in **Table 3.17**) are the same or almost similar for all variables included in both models. The correction of r^2 proves the model to be generalizable beyond the sample. Neither a tendency towards heteroscedasticity nor a deviation from normal distribution of residuals can be observed (for details see **Fig.3.22.**)

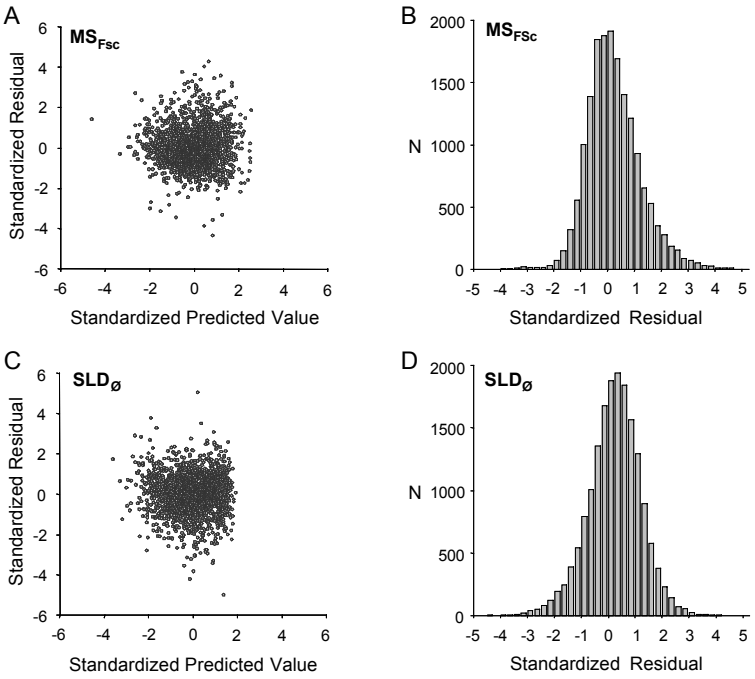


Fig.3.22.: Test for homoscedasticity (A+C) and normal distribution of residuals (B+D).
The standardized residuals (y-axis) are graphed against the standardized predicted values (x-axis). The variance of the standardized residuals should be about the same for all levels of standardized predicted values. No tendency can be observed for both MS_{FSc} (A) and $SLD_{Ø}$ (C), thus, no heteroscedasticity is assumed.
Values of residuals are normally distributed for MS_{FSc} (B) and $SLD_{Ø}$ (D). Kolmogorov-Smirnov test for frequencies of 0.25 bins, MS_{FSc}: p=0.5, $SLD_{Ø}$: p=0.07. A slight skewness towards higher values of standardized predicted values is apparent for MS_{FSc}, while the opposite (skewness towards lower values) can be seen for $SLD_{Ø}$.

Furthermore, Durbin-Watson test indicates no autocorrelation (MS_{FSc} = 1.94, $SLD_{Ø}$ = 1.95). Multicollinearity is not assumed due to values of variance influencing factors (VIF) close or even very close to 1 (average VIF: MS_{FSc} = 1.06, $SLD_{Ø}$ =1.08, **Table 3.17.**).

b) Deviation from normal distribution

Normal distribution of quantitative variables is assumed for regression. ØDOLE follows a Poisson distribution. AGE is skewed towards older ages (ø = 34.1 years, mode = 25 years) and BMI slightly towards higher values of BMI. All variables, including MS_{FSc} and $SLD_{Ø}$, were logarithmically transformed (log10) and tested for normal distribution using the one-sample Kolmogorov-Smirnov test (**Table 3.16.A**). Except for $SLD_{Ø}$, logarithmic transformation improved normality of the distributions. Regression models were tested with transformed quantitative variables for MS_{FSc} (**Table 3.16.B**) and $SLD_{Ø}$ (**Table 3.16.C**). Because MS_{FSc} also differs from normal distribution ($SLD_{Ø}$ is assumed to be normally distributed, see **Table 3.16.A**), the model was separately tested with log10-transformed MS_{FSc} (**Table 3.16.B**). For results and details see **Table 3.16**.

A

	Kolmogorov-Smirnov-Z
AGE	15.15
AGE log10	9.68
ØDOLE	22.66
ØDOLE log10	10.30
BMI	11.99
BMI log10	6.58
MS_{FSc}	8.41
MS_{FSc} log10	5.04
$SLD_{Ø}$	4.43
$SLD_{Ø}$ log10	8.24

C

$SLD_{Ø}$ All ages	No log10 trans.	Logarithmic (Log10) transformation AGE	AGE- ØDOLE	AGE-ØDOLE-BMI
AGE	0.042	0.046	0.046	0.046
GENDER	0.015	0.015	0.015	0.015
ØDOLE	0.004	0.004	0.004	0.004
BMI	0.005	0.004	0.004	0.004

$SLD_{Ø}$ Age > 20	No log10 trans.	Logarithmic (Log10) transformation AGE	AGE- ØDOLE	AGE-ØDOLE-BMI
AGE	0.036	0.040	0.040	0.040
GENDER	0.015	0.015	0.015	0.015
ØDOLE	0.004	0.005	0.004	0.004
BMI	0.005	0.005	0.005	0.005

B

MS_{FSc} All ages	No log10 trans.	Logarithmic (Log10) transformation AGE	AGE- ØDOLE	AGE-ØDOLE-BMI
AGE	0.040	0.038	0.038	0.038
GENDER	0.018	0.018	0.018	0.018
ØDOLE	0.011	0.011	0.014	0.014
PHOTO	0.005	0.004	0.004	0.004
POR	0.016	0.017	0.017	0.017
BMI	0.000	0.000	0.000	0.000

MS_{FSc} Age > 20	No log10 trans.	Logarithmic (Log10) transformation AGE	AGE- ØDOLE	AGE-ØDOLE-BMI
AGE	0.046	0.049	0.049	0.049
GENDER	0.019	0.019	0.019	0.019
ØDOLE	0.012	0.013	0.016	0.016
PHOTO	0.006	0.006	0.005	0.005
POR	0.016	0.016	0.015	0.015
BMI	0.001	0.000	0.001	0.001

MS_{FSc} Log 10 All ages	No log10 trans.	Logarithmic (Log10) transformation AGE	AGE- ØDOLE	AGE-ØDOLE-BMI
AGE	0.038	0.035	0.035	0.035
GENDER	0.014	0.013	0.013	0.013
ØDOLE	0.011	0.012	0.014	0.014
PHOTO	0.004	0.003	0.003	0.003
POR	0.015	0.016	0.016	0.016
BMI	0.001	0.001	0.001	0.001

MS_{FSc} Log 10 Age >20	No log10 trans.	Logarithmic (Log10) transformation AGE	AGE- ØDOLE	AGE-ØDOLE-BMI
AGE	0.046	0.048	0.048	0.048
GENDER	0.014	0.014	0.014	0.014
ØDOLE	0.014	0.014	0.016	0.016
PHOTO	0.005	0.005	0.005	0.005
POR	0.015	0.015	0.015	0.015
BMI	0.001	0.001	0.001	0.001

Table 3.16.:
A) Kolmogorov-Smirnov-Z values for variables before and after log10 transformation. The strongest improvement can be seen for ØDOLE, while MS_{FSc} didn't change much and becomes even worse. Still, after transformation, all distributions significantly deviate from normal distribution (K-S: $p<0.001$). However, this might be a matter of high numbers. When taking a random subsample (n=1000), $SLD_{Ø}$ does not significantly differ from normal distribution (K-S: $p=0.14$) while the other variables still do.

B) Regression models with log10-transformed MS_{FSc} and predictors. Models are represented by the values of **change in r^2**. Left column of the upper left table (**No log10 trans.)** shows results from the initial model (**Table 3.17**). The next column shows results when only AGE is log10-transformed. Then, log10-transformed ØDOLE is added and finally log10 transformed BMI. All steps for MS_{FSc} (left tables) and log10 transformed MS_{FSc} (right tables), respectively. Because of the age-dependent change of trend of MS_{FSc} (see Roenneberg *et al*, 2004), results of models are shown separately for Age > 20 (lower tables).

C) Values of **change in r^2** as described in B) for $SLD_{Ø}$.

All results shown are highly significant ($p<0.001$) except BMI in the models of MS_{FSc} ($p<0.05$).

Variables were subsequently transformed, in the same order of importance as they were included into the model. Logarithmic transformation results in more or less different values of r^2. Most differences are minimal and do not change the order of variables concerning explained variance except for MS_{FSc} log10 where ØDOLE explains slightly more variance than GENDER when AGE & ØDOLE and AGE, ØDOLE, & BMI are also log10 transformed. In summary, log10-transformations do not markedly change values of r^2 (max. change ± 0.003).

c) Age dependency of MS_{FSc} and $SLD_{Ø}$

Taking only Age≥20, however, increases the impact of AGE. This is obvious when looking at the age-dependent change of MS_{FSc} (**Fig.3.23.A**).

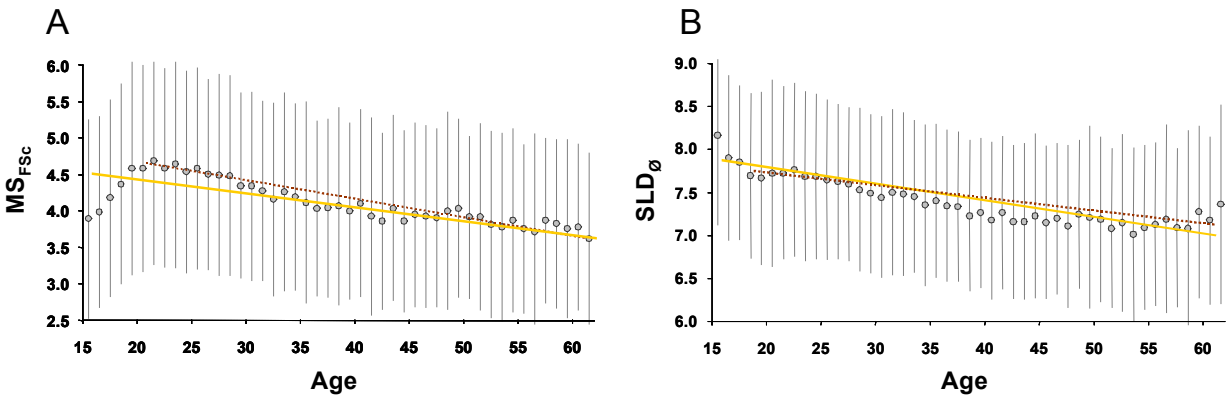


Fig.3.23.:
A) Age dependency of MS_{FSc} (1 year bins), redrawn for the database used for Multiple regression. When estimating a linear regression line through Age ≥ 20 (brown, dotted line), it will be steeper than a regression line through all data points (golden, solid line). Thus, the variance of MS_{FSc} explained by AGE increases when only considering Age ≥20.
B) Because of the stronger decrease of **$SLD_{Ø}$** between 15 and 20 years, the overall regression line (golden, solid line) is steeper than the regression line for Age≥20 (brown, dotted line). The relationship between AGE and $SLD_{Ø}$ could rather be estimated using a non-linear regression equation, however, linear regression is used in this model.

Differences between Age <20 and Age≥20 for $SLD_{Ø}$ are very likely to be due to a not-linear relationship between AGE and $SLD_{Ø}$. Fitting a linear regression results in a steeper regression line when all age groups are considered (**Fig.3.23.B**). The values of r^2 of other variables are not markedly different when including only Age≥20 in the model, neither for MS_{FSc} nor for $SLD_{Ø}$.

For all alternative models with various logarithmically transformed variables, sign and order of B, Beta, and t-values are identical. All coefficients are highly significant for all models ($p<0.001$). Because log10-transformation does not change the model substantially, no transformation will be used for further models. Furthermore, all age groups will be considered together.

3.4.2.3. General regression model

a) MS_{FSc}

All variables, except for LAT, were included into the model. AGE is the strongest predictor for MS_{FSc} (Beta = -0.208) and predicts about 4% of the variance of MS_{FSc}. Using the MCTQ database, Roenneberg *et al* (2004b) were the first to show a systematic and highly quantitative age-dependent change in chronotype on the population level. Also, a reduced sleep consolidation with a wake time at an earlier phase of the body temperature and plasma melatonin rhythm has been reported (Dijk *et al*, 2000). The negative sign indicates that MS_{FSc} decreases (advances) with increasing age.

Adan & Natale (2002) showed women to be earlier types on the Horne-Östberg scale and Roenneberg *et al* (2003) found that MS_F (not MS_{FSc}) occurs later for men than for women (however, in this study the difference was not significant). Here, differences between males and females are highly significant ($p<0.001$) and GENDER even is the second best predictor in the set of variables (Beta = -0.153, 1.82% of variance).

Light is the most powerful zeitgeber for entraining the circadian clock (Pittendrigh & Daan, 1976a; Aschoff & Wever, 1981; Pittendrigh, 1967; Küller, 2002; Panda *et al*, 2002; Czeisler, 1995; Czeisler *et al*, 1986; Jewitt *et al*, 1991; Jewitt *et al*, 1994; Minors *et al*, 1991) and the human circadian clock has been shown to be sensitive to very dim light conditions (Boivin *et al*, 1996; Kronauer *et al*, 1999) and different wavelengths (Thapan *et al*, 2001). Roenneberg *et al* (2003) showed a significant negative correlation between self-reported time spent outside and MS_F. However, no seasonal (photoperiodical) differences were investigated. Their results are consistent with the results of the model, where increasing ØDOLE significantly predicts a decrease of MS_{FSc} ($p>0.001$, Beta = -0.089, 1.06% of variance).

Photoperiod causes changes in reproduction in animals (Cagnacci & Volpe, 1996; Chik *et al*, 1992), is also associated with reproductive changes in humans (Roenneberg & Aschoff, 1990a+b) and with a special form of depression, the Seasonal Affective Disorder (SAD, Wirz-Justice *et al* 2001). Longer photoperiods could be hypothesized to advance sleep times due to a longer availability of sun light each day. MS_{FSc} is, on average, significantly advanced between April and September (compared to the months October to March, $p<0.001$, Beta = -0.067) independent of ØDOLE. Thus, daylength could also be a significant predictor of MS_{FSc} (0.45% of variance).

When assessing the impact of POR, many influences, photic and non-photic, could be involved. Entrainment of circadian rhythms by non-photic zeitgebers has been discussed in several studies (e.g. Honma *et al*, 1994; Klerman, 2001; Klerman *et al*, 1998; Wever, 1979). POR has almost the same capability of predicting MS_{FSc} as GENDER. People living in cities

with more than 100,000 residents on average have a later MS_{FSc} ($p<0.001$, Beta = -0.126, 1.61% of variance).

BMI predicts MS_{FSc} rather weakly but still significantly. Increasing BMI is linked with an advanced MS_{FSc} (0.03% of variance). When comparing value of F-change of BMI with the value of F-change of other variables, BMI does not improve the capabilty of the model to predict MS_{FSc} substantially. Its importance therefore should be taken with care.

b) $SLD_{Ø}$

The results from the model of $SLD_{Ø}$ are different from those of the model of MS_{FSc}. AGE is the strongest predictor of $SLD_{Ø}$ ($p<0.001$, Beta = -0.160, 4.20% of variance) in the way that a shorter $SLD_{Ø}$ comes along with increasing AGE. GENDER is again the second best predictor with women sleeping on average longer than men ($p<0.001$, Beta = 0.190, 1.54% of variance). This has been shown before by Roenneberg *et al* (2003), however, results were significant only for SLD_W.

ØDOLE predicts $SLD_{Ø}$ weaker than MS_{FSc} ($p<0.001$, Beta = -0.059, 0.38% of variance) and a longer ØDOLE is associated with shorter $SLD_{Ø}$.

Longer photoperiods might shorten nocturnal melatonin secretion, possibly leading to shorter $SLD_{Ø}$. The opposite could be hypothesized for shorter photoperiods. Yoneyama *et al* (1999) showed that circadian rhythms in plasma melatonin and rectal temperature but not the sleep-wake-cycle change with photoperiod. However, this study used only nine individuals under extreme conditions (antarctica). Here, a change in PHOTO are not associated with a change in $SLD_{Ø}$.

Latitude has been shown to weakly correlate with urinary melatonin concentrations (Wetterberg *et al*, 1999). This study comprised data from latitudes between 31 degrees South to 77 degrees North. Our data, however, were collected only within a very narrow range of latitude (see 3.4.1.) and like for MS_{Fsc}, LAT is not significantly associated with $SLD_{Ø}$.

POR also does not predict $SLD_{Ø}$ significantly while BMI is stronger associated with $SLD_{Ø}$ than with MS_{FSc} ($p<0.001$, Beta = -0.076, F-change = 94.53, 0.51% of variance). Here, increasing BMI is linked with a shorter $SLD_{Ø}$. A previous study also linked increased BMI to decreased sleep duration (Taheri *et al*, 2004). However, only individuals beyond a BMI value of 30 were included in this study.

Results, represented by the value of explained variance of MS_{FSc} and $SLD_{Ø}$ (change in r^2) are shown in **Table 3.17.** and **Fig.3.24.**

MS_{FSc} (n=16926)		B	Beta	T	Sig.	VIF	cum. r^2	corr. r^2	change r^2	% var.	F change	Sig. F change
	Constant	5.937		95.277	<0.001							
Block 1	AGE	-0.024	-0.208	-26.773	<0.001	1.125	0.040	0.040	0.040	**3.99**	703.146	<0.001
	GENDER	-0.412	-0.153	-20.281	<0.001	1.061	0.058	0.058	0.018	**1.82**	327.251	<0.001
Block 2	ØDOLE	-0.058	-0.089	-11.933	<0.001	1.034	0.069	0.068	0.011	**1.06**	192.116	<0.001
	PHOTO	-0.227	-0.067	-9.116	<0.001	1.018	0.073	0.073	0.005	**0.45**	82.946	<0.001
	LAT *	-----		-----		-----		-----			-----	
Block 3	POR	-0.344	-0.126	-17.169	<0.001	1.008	0.089	0.089	0.016	**1.61**	298.276	<0.001
	BMI	-0.005	-0.019	-2.373	0.018	1.135	0.090	0.089	0.000	**0.03**	5.632	0.018

$SLD_{Ø}$ (n=17313)		B	Beta	T	Sig.	VIF	cum. r^2	corr. r^2	change r^2	% var.	F change	Sig. F change
	Constant	8.250		191.723	<0.001							
Block 1	AGE	-0.013	-0.160	-20.512	<0.001	1.122	0.042	0.042	0.042	**4.20**	759.332	<0.001
	GENDER	0.210	0.109	14.431	<0.001	1.061	0.057	0.057	0.015	**1.54**	282.983	<0.001
Block 2	ØDOLE	-0.028	-0.059	-7.932	<0.001	1.021	0.061	0.061	0.004	**0.38**	70.039	<0.001
	PHOTO *	-----		-----		-----		-----			-----	
	LAT *	-----		-----		-----		-----			-----	
Block 3	POR *	-----		-----		-----		-----			-----	
	BMI	-0.016	-0.076	-9.723	<0.001	1.126	0.066	0.066	0.005	**0.51**	94.529	<0.001

Table 3.17.: Hierarchical multiple regression analyses with stepwise inclusion of predictor (independent) variables. Variables indicated with a (*) were excluded during the analysis due to a lack of significance. Cumulative values of r^2 (cum. r^2) are summed up from top to bottom. Change of r^2 indicates variance of the dependent variables explained by each predictor, also shown as percentage of variance (% var.). Stein's formula was used for adjustment of r^2 (corr. r^2, Field 2000, p.130). For detailed explanations see text.

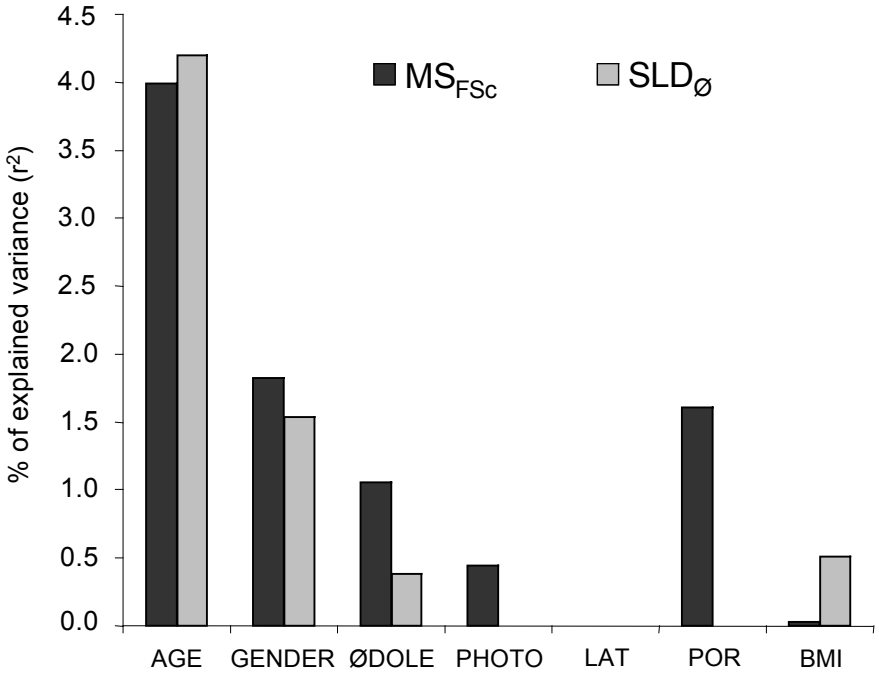


Fig.3.24.: Results presented in **Table 3.17.** for MS_{FSc} and $SLD_{Ø}$. Bars show values of percentage of variance of MS_{FSc} (black bars) and $SLD_{Ø}$ (grey bars) explained by independent variables. If no bar is shown, the respective variable has been excluded from the regression model.

3.4.2.4. Regression models for Work days and for Free days

Note: All models that will be further presented are highly significant (ANOVA for the overall model: $p<0.001$). The models also fulfill all assumptions presented in chapter 3.4.2.2.

The association of predictors with outcomes is tested separately for Work days and for Free days (MS_W and MS_F, SLD_W, and SLD_F). MS_{FSc} is substituted by MS_W and MS_F, respectively. $SLD_Ø$ is replaced by SLD_W and SLD_F. The same conditions as shown in chapter 3.4.2.1. are used.

a) MS_W and MS_F

The general structure of the model is the same as for MS_{FSc}. All variables included are highly significant except for BMI ($p=0.025$ for MS_W and $p=0.04$ for MS_F). LAT is excluded from both models. The negative sign of coefficients (B, Beta, and T) indicates the same association of predictors with MS_W and MS_F as with MS_{FSc}, e.g. with increasing AGE, MS_W and MS_F decrease. The amount of variance of MS_W and MS_F explained by predictors, however, differs from MS_{FSc}.

The largest difference between the models is the variance of the outcomes (MS_W and MS_F) explained by AGE. Almost 15% of the variance of MS_F can be explained by AGE whereas the association with MS_W is much weaker (1.75% of explained variance).

POR is a stronger predictor for MS_W (about 3% of explained variance) than for MS_F (about 1% of explained variance). GENDER shows a slightly stronger association with MS_F (1.83%, MSW: 1.48%) and ØDOLE predicts MS_W (1.82%) better than MS_F (0.81%). No substantial difference can be observed for PHOTO (MS_W: 0.42%, MS_F: 0.59%) and BMI (MS_W: 0.03%, MSF: 0.02%) and the contribution of BMI is very weak compared to the other variables. As already mentioned for the model of MS_{FSc}, BMI's contribution to the models is rather doubtful when comparing values of F-changes. Results are shown in **Table 3.18.** and **Fig.3.25a.**

MS_W	(n = 17131)	B	Beta	T	Sig.	VIF	cum. r^2	corr. r^2	change r^2	% var.	F change	Sig. F change
	Constant	4.406		88.910	<0.001							
Block 1	**AGE**	-0.013	-0.135	-17.475	<0.001	1.122	0.017	0.017	0.017	**1.75**	304.989	<0.001
	GENDER	-0.308	-0.144	-19.154	<0.001	1.059	0.032	0.032	0.015	**1.48**	261.420	<0.001
Block 2	**ØDOLE**	-0.062	-0.120	-16.092	<0.001	1.033	0.051	0.050	0.018	**1.82**	329.161	<0.001
	PHOTO	-0.173	-0.065	-8.786	<0.001	1.017	0.055	0.054	0.004	**0.42**	75.543	<0.001
Block 3	**POR**	-0.374	-0.173	-23.525	<0.001	1.008	0.085	0.084	0.030	**2.98**	558.377	<0.001
	BMI	-0.004	-0.018	-2.248	0.025	1.135	0.085	0.084	0.000	**0.03**	5.054	0.025

MS_F	(n = 16919)	B	Beta	T	Sig.	VIF	cum. r^2	corr. r^2	change r^2	% var.	F change	Sig. F change
	Constant	7.188		112.637	<0.001							
Block 1	**AGE**	-0.050	-0.395	-53.740	<0.001	1.125	0.147	0.147	0.147	**14.67**	2907.658	<0.001
	GENDER	-0.440	-0.151	-21.182	<0.001	1.062	0.165	0.165	0.018	**1.83**	369.858	<0.001
Block 2	**ØDOLE**	-0.054	-0.077	-10.922	<0.001	1.034	0.173	0.173	0.008	**0.81**	166.708	<0.001
	PHOTO	-0.281	-0.077	-11.018	<0.001	1.017	0.179	0.179	0.006	**0.59**	121.322	<0.001
Block 3	**POR**	-0.299	-0.101	-14.551	<0.001	1.008	0.189	0.189	0.010	**1.03**	214.318	<0.001
	BMI	-0.005	-0.015	-2.051	0.040	1.135	0.189	0.189	0.000	**0.02**	4.209	0.040

SLD_W	(n = 17144)	B	Beta	T	Sig.	VIF	cum. r^2	corr. r^2	change r^2	% var.	F change	Sig. F change
	Constant	7.694		161.456	<0.001							
Block 1	**AGE**	-0.006	-0.071	-8.963	<0.001	1.123	0.033	0.033	0.008	**0.85**	150.608	<0.001
	GENDER	0.254	0.127	16.434	<0.001	1.059	0.025	0.025	0.025	**2.46**	432.383	<0.001
Block 2	**ØDOLE**	-0.026	-0.053	-6.941	<0.001	1.033	0.036	0.036	0.003	**0.29**	52.401	<0.001
	PHOTO	0.057	0.023	2.988	0.003	1.017	0.036	0.036	0.000	**0.03**	5.571	0.018
Block 3	**POR**	-0.050	-0.025	-3.266	0.001	1.008	0.040	0.040	0.001	**0.06**	10.669	0.001
	BMI	-0.014	-0.066	-8.238	<0.001	1.135	0.041	0.041	0.004	**0.39**	70.109	<0.001

SLD_F	(n = 16932)	B	Beta	T	Sig.	VIF	cum. r^2	corr. r^2	change r^2	% var.	F change	Sig. F change
	Constant	9.690		137.743	<0.001							
Block 1	**AGE**	-0.032	-0.248	-31.792	<0.001	1.124	0.075	0.075	0.075	**7.46**	1364.772	<0.001
	GENDER	0.107	0.036	4.687	<0.001	1.061	0.077	0.077	0.002	**0.22**	39.855	<0.001
Block 2	**ØDOLE**	-0.031	-0.042	-5.627	<0.001	1.034	0.079	0.079	0.002	**0.21**	38.559	<0.001
	PHOTO	-0.111	-0.029	-3.966	<0.001	1.017	0.080	0.080	0.001	**0.11**	21.042	<0.001
Block 3	**POR**	0.081	0.026	3.564	<0.001	1.008	0.083	0.083	0.001	**0.07**	12.699	<0.001
	BMI	-0.019	-0.059	-7.501	<0.001	1.134	0.084	0.083	0.003	**0.29**	54.236	<0.001

Table 3.18.: Results of regression models for the outcomes MS_W, MS_F, SLD_W, and SLD_F (as presented in **Table 3.17.**). For all models, LAT was excluded from the model is not shown here. For explanations see **Table 3.17.**

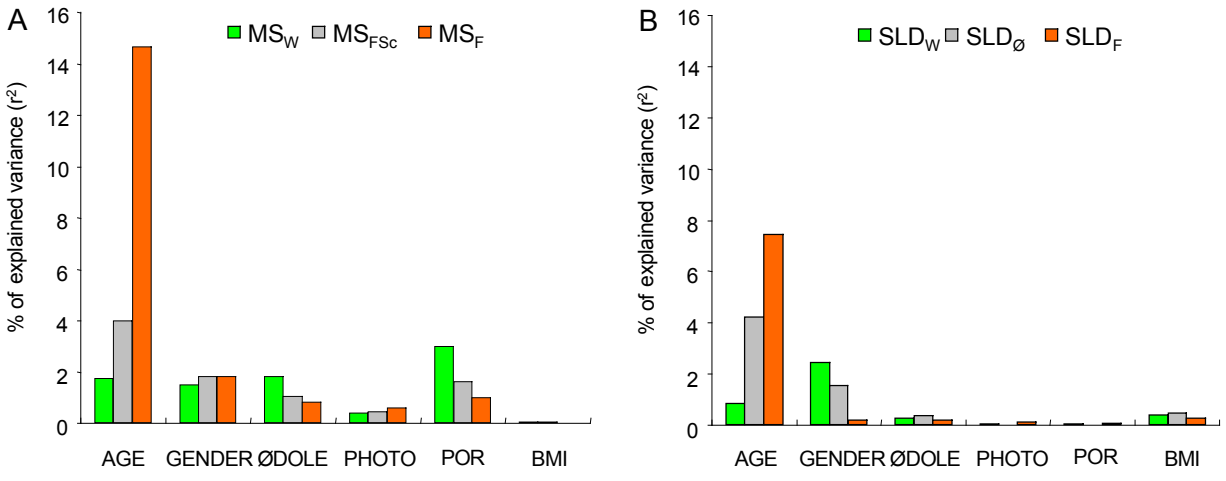


Fig.3.25.: Graphs of regression models for the outcomes MS_W & MS_F (A) and SLD_W & SLD_F (B), represented by values of change in r^2 (green bars: work days, orange bars: free days; **Table 3.18.**). Values of change in r^2 for MS_{FSc} and $SLD_Ø$ are respectively shown as grey bars (for details see **Table 3.17.**).

b) SLD_W and SLD_F

As shown in **Table 3.18.**, the amount of variance explained by AGE is much higher for SLD_F(7.46%) than for SLD_W (0.85%). Substantial differences are also apparent for GENDER (SLD_W: 2.46%, SLD_F: 0.22%). In contrast to the model of $SLD_Ø$, PHOTO and POR are included into the models of SLD_W and SLD_F. The months from April to September are linked to a shorter sleep duration on free days and a longer sleep duration on work days. PORs with less than 100,000 habitants are linked to a longer sleep duration on free days and a shorter sleep duration on work days. PHOTO and POR are only weakly associated with both SLD_W and SLD_F; calculating $SLD_Ø$ eliminates these weak associations. Variance explained by Light and BMI is not markedly different between SLD_W (Light: 0.29%, BMI: 0.39%) and SLD_F (ØDOLE: 0.21%, BMI: 0.29%) and for both, ØDOLE and BMI, higher for $SLD_Ø$ (Light: 0.38%, BMI: 0.51%). All results are also graphically shown in **Fig.3.25b.**

c) Summary for differences between work days and free days

In general, the amount of variance explained by the predictors is rather small for the models of MS_{FSc} and $SLD_Ø$. But, AGE explains 14.67% of variance of MS_F and 7.46% of variance of SLD_F. MS_F and SLD_F are strongly influenced by AGE, whereas the influence on MS_W and SLD_W is small (1.75% and 0.85%, respectively). In contrast, POR shows a three fold higher association with MS_W (2.98%) than with MS_F (1.03%) while the association of POR with SLD_W and SLD_F is very small (0.06% and 0.07%, respectively) and not apparent for $SLD_Ø$. The association of POR with SLD_W and SLD_F is neglectably small but, interestingly, PORs with less than 100 000 habitants are linked to shorter SLD_W and longer SLD_F.

All in all, MS_{FSc} and $SLD_Ø$ not only seem to be independent of each other (see chapter 3.3.) but also different in the way their variance can be explained by biological and social factors. $SLD_Ø$ is markedly influenced only by AGE and GENDER and SLD_F is strongly influenced only by AGE (**Fig.3.25.**). Besides AGE and GENDER, an association of MS_{FSc} with ØDOLE (1.06%) and POR (1.61%) is worth mentioning. For all phase markers (MS_W, MS_F, and MS_{FSc}), an increased amount of ØDOLE and the fact of living in a POR with less than 100,000 residents is linked to an advanced Mid-sleep. For both of these predictors, the association with MS_W (Light: 1.82%, City: 2.98%) is stronger than with MS_F (Light: 0.81%, City: 1.03%).

Roenneberg *et al* (2003a) mentioned that daily sleeping patterns are influenced by three different clocks: i) the social clock, ii) the solar clock, iii) and the circadian clock.

"Social clock" is a very comprehensive term and could include any environmental influence apart from the solar and biological ones. Chapter 3.3. showed that SE_W has an impact on

other parameters, both on work days and on free days. The influence of SE_W, however, is almost not apparent for MS_{FSc} and $SLD_Ø$ (MS_{FSc} and $SLD_Ø$ load only weakly on the factor 'Social jet lag'; Factor 3 in **Table 3.14.**) indicating an independence of MS_{FSc} and $SLD_Ø$ from this "part" of the social clock.

From many studies, light is known to be the strongest entraining signal for the circadian clock but results were obtained under laboratory conditions (Czeisler 1995; Czeisler *et al*, 1986; Jewitt *et al*, 1991; Jewitt *et al*, 1994; Minors *et al*, 1991). In this study, ØDOLE is a social factor because people expose themselves, for social reasons, more or less to daylight. The association of ØDOLE on MS_{FSc} appears to be small (1.06%, MS_W: 1.82%, MS_F: 0.81%). The MCTQ only assesses total time spent outside, not the exact timing of outside light exposure. The effect of light on the circadian clock, however, crucially depends on internal phase, shown by PRC (see chapter 1.3.2.2.).

The association of PHOTO with MS_{FSc} is weak (0.45%) and about the same for MS_W (0.42%) and MS_F (0.59%). PHOTO is exluded from the model of $SLD_Ø$, but the summer months (April to September) are very weakly linked to longer SLD_W (0.03) and shorter SLD_F (0.11%).

All variance not explained by (not clock related) biological and social factors should be addressed to properties of the circadian clock. Of course, only a part of all possible influences are considered here, be it that they are not assessable by the MCTQ or that they are not even known by now. Therefore, one has to cautious when applying any amount of variance of MS_{FSc} and $SLD_Ø$ to a biological clock.

3.4.2.5. Regression models for Age groups and Gender

In previous regression models, AGE and GENDER were shown to have the strongest association with MS_{FSc} and $SLD_{Ø}$. Regression models werr also conducted separately for Females, Males, Age≤30, and Age>30. Conditions and variables are the same as shown in chapter 3.4.1.+2. The variables AGE and GENDER are excluded when comparing models for the respective variable. Results of all models are shown in **Table 3.19.** and **Fig.3.26.**

MS_{FSc}	<30 (n=8234)	Beta	T	Sig.	corr. r^2	change r^2	% var.	F change	Sig. F change
	Constant		117.702	<0.001					
Block 1	**AGE**		----			----		----	
	GENDER	-0.193	-17.986	<0.001	0.037	0.037	**3.69**	315.163	<0.001
Block 2	**ØDOLE**	-0.043	-3.965	<0.001	0.043	0.002	**0.24**	21.021	<0.001
	PHOTO	-0.082	-7.602	<0.001	0.046	0.007	**0.68**	58.720	<0.001
Block 3	**POR**	-0.123	-11.480	<0.001	0.061	0.015	**1.50**	131.785	<0.001
	BMI		-----			-----		-----	

MS_{FSc}	Female (n=8688)	Beta	T	Sig.	corr. r^2	change r^2	% var.	F change	Sig. F change
	Constant		90.126	<0.001					
Block 1	**AGE**	-0.149	-14.113	<0.001	0.021	0.022	**2.16**	191.668	<0.001
	GENDER		----			----		----	
Block 2	**ØDOLE**	-0.051	-4.820	<0.001	0.025	0.003	**0.34**	30.343	<0.001
	PHOTO	-0.032	-3.058	0.002	0.025	0.001	**0.08**	7.444	0.006
Block 3	**POR**	-0.126	-11.968	<0.001	0.041	0.016	**1.58**	143.232	<0.001
	BMI		-----			-----		-----	

MS_{FSc}	>30 (n=8692)	Beta	T	Sig.	corr. r^2	change r^2	% var.	F change	Sig. F change
	Constant		59.412	<0.001					
Block 1	**AGE**		----			----		----	
	GENDER	-0.097	-9.051	<0.001	0.004	0.004	**0.37**	32.319	<0.001
Block 2	**ØDOLE**	-0.137	-12.765	<0.001	0.026	0.023	**2.28**	203.910	<0.001
	PHOTO	-0.055	-5.238	<0.001	0.030	0.003	**0.34**	30.638	<0.001
Block 3	**POR**	-0.129	-12.277	<0.001	0.046	0.017	**1.69**	154.008	<0.001
	BMI	-0.034	-3.223	0.001	0.047	0.001	**0.11**	10.387	0.001

MS_{FSc}	Male (n=8238)	Beta	T	Sig.	corr. r^2	change r^2	% var.	F change	Sig. F change
	Constant		109.229	<0.001					
Block 1	**AGE**	-0.255	-24.466	<0.001	0.078	0.078	**7.78**	694.853	<0.001
	GENDER		----			----		----	
Block 2	**ØDOLE**	-0.108	-10.294	<0.001	0.093	0.016	**1.58**	143.268	<0.001
	PHOTO	-0.099	-9.548	<0.001	0.103	0.010	**1.00**	91.680	<0.001
Block 3	**POR**	-0.127	-12.210	<0.001	0.119	0.016	**1.59**	149.072	<0.001
	BMI		-----			-----		-----	

$SLD_{Ø}$	<30 (n=8378)	Beta	T	Sig.	corr. r^2	change r^2	% var.	F change	Sig. F change
	Constant		124.328	<0.001					
Block 1	**AGE**		----			----		----	
	GENDER	0.141	12.943	<0.001	0.023	0.023	**2.32**	199.109	<0.001
Block 2	**ØDOLE**	-0.056	-5.219	<0.001	0.026	0.003	**0.33**	28.071	<0.001
	PHOTO		-----			-----		-----	
	LAT		-----			-----		-----	
Block 3	**POR**		-----			-----		-----	
	BMI	-0.063	-5.782	<0.001	0.030	0.004	**0.39**	33.427	<0.001

$SLD_{Ø}$	Female (n=8884)	Beta	T	Sig.	corr. r^2	change r^2	% var.	F change	Sig. F change
	Constant		160.169	<0.001					
Block 1	**AGE**	-0.199	-18.612	<0.001	0.046	0.046	**4.62**	430.617	<0.001
	GENDER		----			----		----	
Block 2	**ØDOLE**	-0.061	-5.862	<0.001	0.050	0.004	**0.42**	39.261	<0.001
	PHOTO		-----			-----		-----	
	LAT		-----			-----		-----	
Block 3	**POR**		-----			-----		-----	
	BMI	-0.079	-7.450	<0.001	0.056	0.006	**0.59**	55.510	<0.001

$SLD_{Ø}$	>30 (n=8935)	Beta	T	Sig.	corr. r^2	change r^2	% var.	F change	Sig. F change
	Constant		129.331	<0.001					
Block 1	**AGE**		----			----		----	
	GENDER	0.094	8.784	<0.001	0.015	0.015	**1.47**	132.963	<0.001
Block 2	**ØDOLE**	-0.069	-6.529	<0.001	0.020	0.006	**0.58**	53.316	<0.001
	PHOTO		-----			-----		-----	
	LAT	0.020	1.961	0.050	0.021	0.000	**0.05**	4.475	0.034
Block 3	**POR**		-----			-----		-----	
	BMI	-0.093	-8.728	<0.001	0.029	0.008	**0.83**	76.170	<0.001

$SLD_{Ø}$	Male (n=8429)	Beta	T	Sig.	corr. r^2	change r^2	% var.	F change	Sig. F change
	Constant		127.448	<0.001					
Block 1	**AGE**	-0.126	-11.058	<0.001	0.024	0.024	**2.45**	211.388	<0.001
	GENDER		----			----		----	
Block 2	**ØDOLE**	-0.066	-6.095	<0.001	0.029	0.005	**0.46**	40.243	<0.001
	PHOTO		-----			-----		-----	
	LAT		-----			-----		-----	
Block 3	**POR**		-----			-----		-----	
	BMI	-0.071	-6.257	<0.001	0.033	0.004	**0.45**	39.147	<0.001

Table 3.19.: Results of regression models for MS_{FSc} and $SLD_{Ø}$, respectively for Females, Males, Age ≤ 30, and Age>30. Values of B, cum. r^2, and VIF are not shown. For explanations see **Table 3.17.**

Note: Results of models presented next cannot be directly compared with results from previous models because either AGE or GENDER were excluded and the effect of these variables is not considered.

a) MS_{FSc}

Differences are evident when analyzing Age groups and Gender separately. AGE explains a 3.5 fold higher amount of variance of MS_{FSc} for Males (7.78%) than for Females 2.16%). The association of GENDER with MS_{FSc} is much higher for Age≤30 (3.69%) than for Age>30 (0.37%).

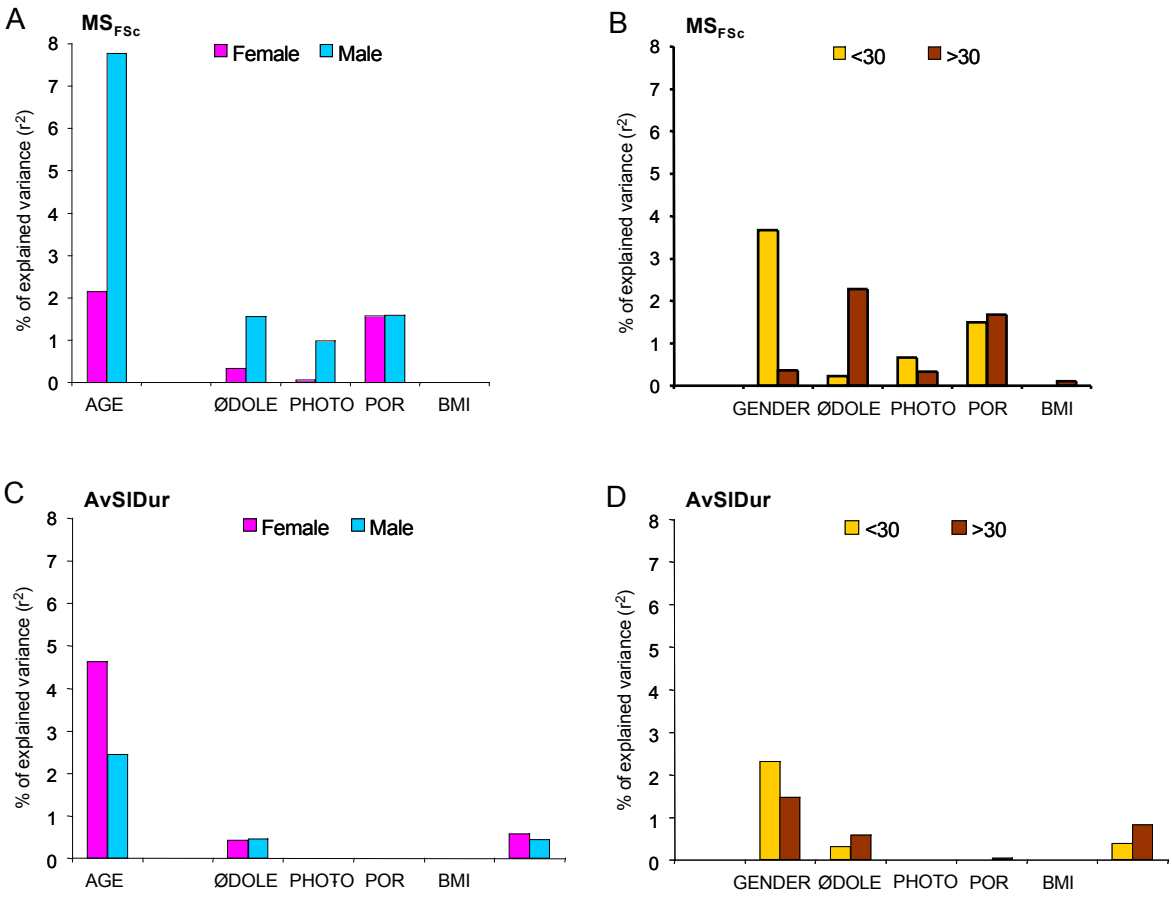


Fig.3.26.: Differences between models shown in **Table 3.18.** represented by values of change in $\mathbf{r^2}$ (pink bars: Females, blue bars: Males, golden bars: Age ≤ 30 years, brown bars: Age>30 years). For details see **Table 3.17.** and text.

Both outcomes reflect the observations of Roenneberg et al (2004, Fig.1d). Between 15 and 50 years of age, males are on average later types (higher MS_{FSc}) than females. The maximum difference is reached at about the age of 20 years, then decreasing constantly until fading out at about the age of 50. The range of average MS_{FSc} throughout life is wider for males, thus age explains a higher amount of variance of MS_{FSc} for males. The average difference of MS_{FSc} between males and females is higher for Age≤30 than for Age>30, the association of GENDER is therefore higher for the first age group.

Increased ØDOLE is linked to advanced MS_{FSc} for all groups. The association with MS_{FSc} is higher for Males (1.58%, Females: 0.34%) and Age>30 (2.28%, Age≤30: 0.24%). Longer PHOTO is also linked to advanced MS_{FSc} for all groups. Almost no association is apparent for Females (0.08%, Males: 1.00%) and Age≤30 is stronger associated with day length (0.68%, Age>30: 0.34%).

There are almost no differenes between groups for POR (Females: 1.58%, Males: 1.59%, Age≤30 years: 1.50%, Age>30 years: 1.69%) and finally, BMI is very weakly associated only with Age>30 but with no other group.

b) $SLD_{Ø}$

AGE explains a higher amount of variance of $SLD_{Ø}$ for Females (4.62%, Males: 2.45%), and being female is more linked to a longer $SLD_{Ø}$ for Age≤30 (2.32%, Age>30: 1.47%). The difference of $SLD_{Ø}$ between females and males is greater in younger ages.

Almost no difference is apparent for ØDOLE between Males (0.42%) and Females (0.46%) wheras an increased amount of ØDOLE is stronger associated with shorter $SLD_{Ø}$ for Age>30 (0.58%, Age≤30: 0.33%). Lower LAT are very weakly linked to shorter $SLD_{Ø}$ only for Age>30 (0.05%) but not with any other group.

BMI is generally associated with shorter $SLD_{Ø}$. There are only small differences between Females (0.59%) and Males (0.45). For Age>30, BMI explains about twice as much variance of $SLD_{Ø}$ (0.83%) than Age≤30 (0.39%).

c) Summary for Age and Gender differences

Variation within MS_{FSc} and $SLD_{Ø}$ associated with AGE and GENDER are different. While AGE can explain a 3.5 fold higher amount of variance of MS_{FSc} for Males than for Females, it explains a smaller amount of variance of $SLD_{Ø}$ for Males. The association of ØDOLE and PHOTO is different between Females and Males only for MS_{FSc} and there are no or only slight differences for $SLD_{Ø}$ (PHOTO is completely excluded from the model of $SLD_{Ø}$). A very weak association of LAT with $SLD_{Ø}$ appears for Age>30. The contribution of LAT to the model of $SLD_{Ø}$ (Age>30) is questionable when looking at value of F-change (=4.48).

All in all, MS_{FSc} is more associated with biological and social factors than $SLD_{Ø}$ and differences between Age groups and Gender are higher for MS_{FSc}. Contributions of predictors to the models are highly significant (F-change > 20, $p<0.001$) exept for BMI for MS_{FSc} (Age≤30, F-change = 10.39, $p=0.001$), PHOTO for MS_{FSc} (Females, F-change = 7.44, $p=0.006$), and LAT for $SLD_{Ø}$ (Age>30, F-change = 4.48, $p=0.036$). The amount of explained variance of the respective outcome is very small and the contribution to the respective model is very weak (PHOTO) or not even apparent (BMI and LAT). An exclusion of these variables from the respective model should be considered.

3.4.2.6. Conclusions

Results shown in chapter 3.4.2. give rise to two major assumptions: i) The association with biological and social factors is different for MS_{FSc} and $SLD_{Ø}$ and ii) association is different when separately analyzing Work days, Free days, Gender, and Age groups.

More interesting than absolute values, e.g. the amount of variance of either MS_{FSc} or $SLD_{Ø}$ explained by a certain variable, is the ratio of respective values when comparing different groups. Between MS_{FSc} and $SLD_{Ø}$, contributions of AGE and GENDER are more or less the same. The associtation of AGE with MS_{FSc} is difficult to assess because of the change in trend of regression line (see Roenneberg *et al*, 2004b, Fig.1c). However, there are no big differences in outcomes when including only Age>20 into regression models (**Table 3.16.** and **Fig.3.23.**). Except for Latitude, which is excluded from both models, results are different for models of MS_{FSc} and $SLD_{Ø}$.

Separate analysis of work days and free days reveals big differences for some variables. The association of AGE is much stronger for MS_F & SLD_F than for MS_W & SLD_W. The association of GENDER is much stronger with SLD_W than with SLD_F but not different between MS_W & MS_F. ØDOLE and POR are also more associated with MS_W and plays no role for SLD_W & SLD_F. Except for AGE, results are very different between MS_{FSc} and $SLD_{Ø}$.

3.4.3. Interaction of factors

3.4.3.1. Objectives

Chapter 3.4.2. revealed an association of MS_{FSc} and $SLD_{Ø}$ with different biological and social factors. Also, direction and, to a certain extent, strength of association could be determined, separately for Gender and Age groups. What remains open is the association between factors (predictors) and a quantitative presentation of results (concrete values of MS_{FSc} and $SLD_{Ø}$ for sub-groups). Analysis of covariance (ANCOVA) is used to determine the position of each sub-group within the distributions of MS_{FSc} and $SLD_{Ø}$.

3.4.3.2. Sub-grouping the sample population and model of ANCOVA

Four factorial Analysis of covariance (ANCOVA) was performed for MS_{FSc} and $SLD_{Ø}$. The factors were (in order of inclusion): POR, AGE, PHOTO, and GENDER. **Fig.3.27.** shows the subsequent division of groups. ØDOLE and BMI were used as covariates, meaning that results are corrected for the amount of light or BMI and (mathematically) independent of these factors.

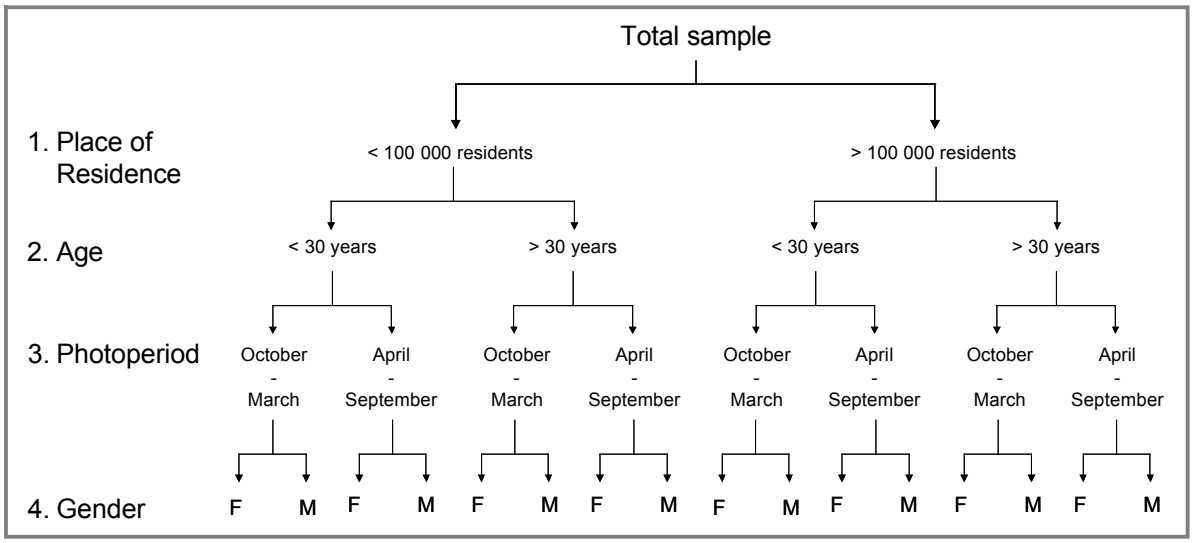


Fig.3.27: By the order of inclusion into the model, the sample population was first separated for POR. Then, both groups (<100,000 residents and >100,000 residents) were each separated for Age (≤30 or >30) and so on. This finally results in 16 different sub-groups (Step 4.). This procedure was performed both for MS_{FSc} and $SLD_{Ø}$.

3.4.3.3. (ln) Accuracy of model

There are four test assumptions for ANCOVA: 1) Normal distribution of quantitative variables, 2) Homogeneity of group variances, 3) Equal number of cases in every group, and 4) Homogeneity of regression slopes (for each group, e.g. females and males, the regression slope 'covariate – outcome' has to be ± equal). In the model, there are eight combinations (AGE-BMI, Age-ØDOLE, GENDER-BMI,…).

From results shown in **Table 3.16.**, MS_{FSc}, $SLD_{Ø}$, ØDOLE and BMI are assumed to be normally distributed because logarithmic transformation did not alter results substantially. However, assumptions 2) to 4) are violated in the model: 2) Levene's test of equality of error variances is significant, both for MS_{FSc} ($F=18.31$, $p<0.001$) and $SLD_{Ø}$ ($F=3.17$, $p<0.001$). When running the model with a random sub sample (5%, n=940), MS_{FSc} is only significant on the 0.05 level ($F=1.84$, $p=0.026$) and $SLD_{Ø}$ is not significant ($F=0.61$, $p=0.87$). 3) There are also differences in number of cases of different groups, ranging from n=310 to n=2466 (MS_{FSc}) and n=319 to n=2513 ($SLD_{Ø}$). Still, even small groups contain a relatively high number of cases. 4) Five of eight covariate by outcome interactions are significant (regression slopes are significantly different) for MS_{FSc} (GENDER-BMI, AGE-BMI, POR-BMI, PHOTO-BMI, AGE-ØDOLE, $p<0.001$) and three of eight interactions are significant for $SLD_{Ø}$ (GENDER -BMI, $p<0.001$; AGE-BMI, $p<0.001$; POR-ØDOLE, $p=0.21$).

Because of the violation of assumptions 2) to 4), extremely stringent conditions are applied: Results are assessed significant only on the 0.01% level ($p<0.0001$).

3.4.3.4. Effects and interactions between independent variables

Four independent variables were included into the model, AGE (≤30 years, >30 years), GENDER, PHOTO (Apr-Sept, Oct-Mar), and POR (<100,000 residents, >100,000 residents), ØDOLE and BMI were included as covariates. Chapter 3.4.3. showed all predictors to be significant for MS_{FSc} but only AGE and GENDER to significantly predict $SLD_{Ø}$. The independent contribution of each variable has been determined (change in r^2). Now the question arises if there is any interaction between two or more of these predictors.

Table 3.20. shows effects of predictors and covariates and the interactions between predictors. As already shown in chapter 3.4.2., AGE and GENDER have the strongest effects on MS_{FSc} and $SLD_{Ø}$. PHOTO and POR have an effect on MS_{FSc} but not on $SLD_{Ø}$. Both covariates have significant effects on $SLD_{Ø}$, only ØDOLE has a significant effect on MS_{FSc}.

		MS_{FSc}		$SLD_{Ø}$	
		F-value	Sig. (p)	F-value	Sig. (p)
	Corrected Model	101.85	**<0.0001**	73.00	**<0.0001**
	Constant	6778.32	**<0.0001**	37389.86	**<0.0001**
Covariates	**BMI**	10.00	**0.002**	**106.52**	**<0.0001**
	ØDOLE	**143.80**	**<0.0001**	**69.32**	**<0.0001**
Factors	**POR**	**202.69**	**<0.0001**	0.01	**0.928**
	AGE	**458.87**	**<0.0001**	**236.21**	**<0.0001**
	PHOTO	**93.67**	**<0.0001**	0.49	**0.486**
	GENDER	**368.98**	**<0.0001**	**168.10**	**<0.0001**
Interactions between factors	**GENDER + AGE**	**62.31**	**<0.0001**	9.41	**0.002**
	GENDER + POR	3.56	**0.059**	2.09	**0.148**
	AGE + POR	3.11	**0.078**	0.00	**0.984**
	GENDER + AGE + POR	0.73	**0.391**	2.11	**0.146**
	GENDER + PHOTO	**38.33**	**<0.0001**	2.29	**0.130**
	AGE + PHOTO	2.33	**0.127**	1.66	**0.198**
	GENDER + AGE + PHOTO	6.55	**0.011**	0.90	**0.344**
	POR + PHOTO	3.09	**0.079**	1.12	**0.290**
	GENDER + POR + PHOTO	0.83	**0.363**	0.04	**0.844**
	AGE + POR + PHOTO	8.75	**0.003**	0.00	**0.978**
	GENDER+AGE+POR+PHOTO	0.04	**0.834**	0.09	**0.767**

Table 3.20.: Effects of predictors, covariates and interactions between predictors for MS_{FSc} and $SLD_{Ø}$. P-values of variables and interactions significant on the 0.01% level (p<0.0001) are shown in green (the respective F-value is shown as bold number). Red numbers indicate p-values (≥0.0001) of not significant predictors and interactions.

Results of groups (**Fig.3.27.**) are graphically presented in **Fig.3.28.A+B**. Following the separation procedure in **Fig.3.27.**, first the total sample is splitted for **POR**, meaning whether someone lives in a city/town/village with more or less than 100,000 residents. This effect is significant for MS_{FSc} (F=202.69, p<0.001) but not for $SLD_{Ø}$ (F=0.01, p=0.928). The effect of POR is shown as grey (>100,000 residents) and green (<100,000 residents) bars in **Fig.3.28.A+B**. There is no interaction between POR and any other predictor, neither for MS_{FSc} nor for $SLD_{Ø}$. This means that the effect of POR does not depend on the effect of another variable.

The effect of **AGE** is significant for both MS_{FSc} (F=458.80, p<0.001) and $SLD_{Ø}$ (F=236.21, p<0.001). A significant interaction between **GENDER** and **AGE** only exists for MS_{FSc} (F=62.31, p<0.001). $SLD_{Ø}$ slightly fails significance due to stringent conditions (F=9.41, p=0.002). The interactions between **GENDER** and **AGE** are graphically shown in **Fig.3.28.C+D**. All Males ≤30 (left blue ellipse), all Males >30 (right blue ellipse), all Females ≤30 (left pink ellipse), and all Females >30 (right pink ellipse) are summarized. Blue and pink dots represent the average values of MS_{FSc} (**Fig.3.28.C**) and $SLD_{Ø}$ (**Fig.3.28.D**) for each summarized group. For MS_{FSc}, the line through average values of Males ≤30 and Males >30 is not parallel to the line through average values of Females ≤30 and Females >30. The difference between Age ≤30 and Age >30 is not the same for Males an Females. An interaction is not apparent for $SLD_{Ø}$ (lines are parallel). Roenneberg *et al* (2004b, Fig.1d) showed that the average MS_{FSc} changes throughout life and that this change is different for females and males.

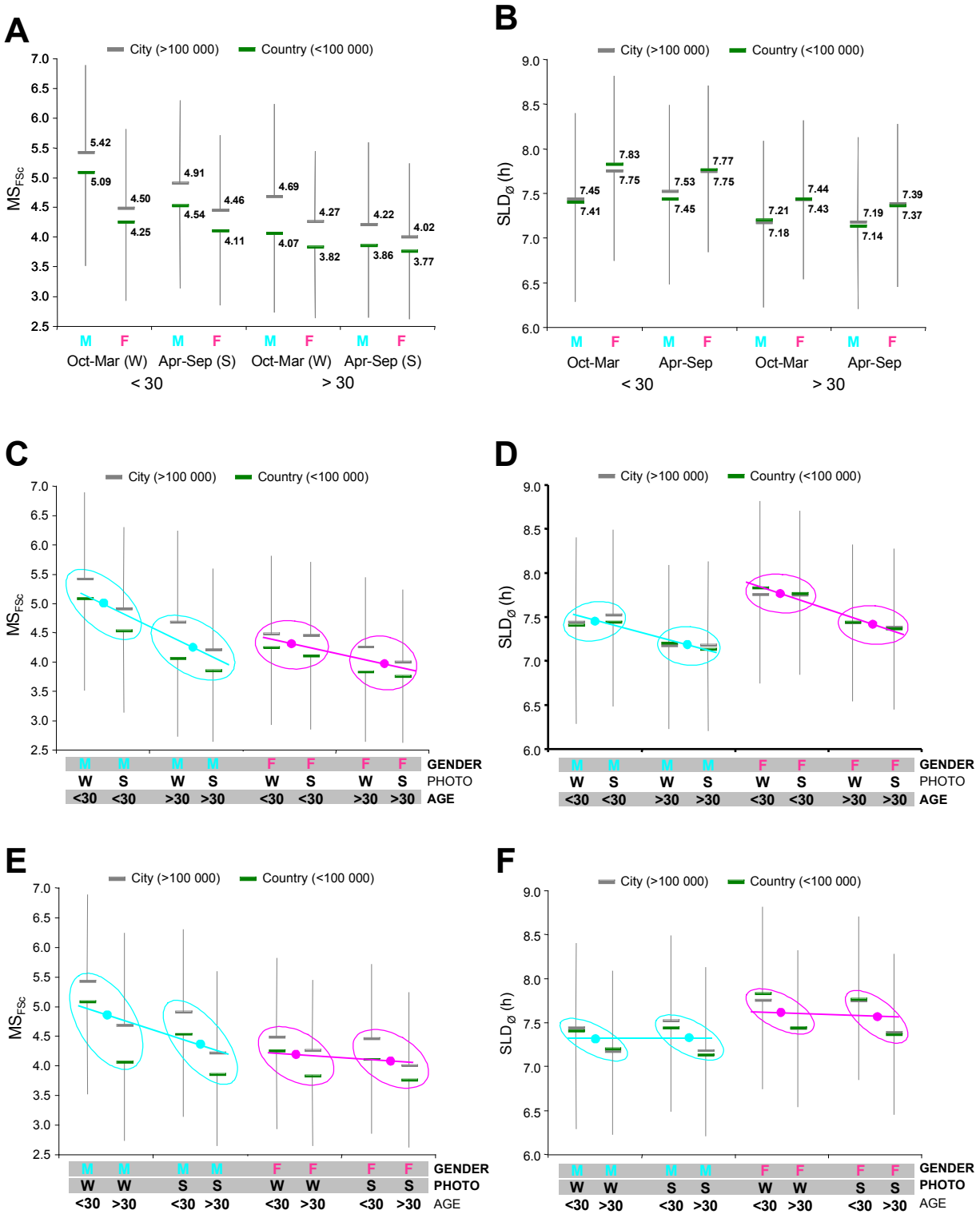


Fig.3.28.:
A+B: Absolut values of groups described in **Fig.3.27.** for MS_{FSc} (A) and $SLD_{Ø}$ (B). Values are corrected for ØDOLE and BMI. Grey bars indicate POR >100,000 (+ standard deviation), green bars represent POR <100,000 (- standard deviation) .
C-F: Interactions of predictors (C+D: GENDER-AGE, E+F: GENDER-PHOTO) are shown as pink and blue lines. Pink and blue dots represent average values of subgroups indicated by pink and blue ellipses. Interacting predictors are emphasized by grey (C+D: GENDER-AGE interactions, E+F: GENDER-PHOTO interactions). W: winter months from October to March; S: summer months from April to September. For explanations see text.

During adolescence, MS_{FSc} of males delays more than MS_{FSc} of females. At about the age of 20 years, the difference is maximal and then declines constantly until it disappears at about the age of 50 years. Here, the interaction of Gender and Age reflects the decreasing difference of MS_{FSc} between females and males.

PHOTO has a significant effect only on MS_{FSc} ($F=93.67$, $p<0.001$, $SLD_{Ø}$: $F=0.49$, $p=0.486$). There is also a significant interaction between **GENDER** and **PHOTO** ($F=38.33$, $p<0.001$) which is graphically represented in **Fig.3.28.E+F**. All Males in the winther months (W, Oct-Mar, left blue ellipse), all Males in the summer months (S, Apr-Sep, right blue ellipse), all Females in winter (left pink ellipse), and all Females in summer (right pink ellipse) are summarized. Analogous to **Fig.3.28.C+D**, there is an interaction between **GENDER** and **PHOTO** for MS_{FSc} but not for $SLD_{Ø}$. The difference of average values of MS_{FSc} between the winter months (W, Oct-Mar) and the summer months (S, Apr-Sep) is greater for Males than for Females.

The effect of **GENDER** is significant for both MS_{FSc} ($F=368.98$, $p<0.001$) and $SLD_{Ø}$ ($F=168.10$, $p<0.001$). There is an interaction between **PHOTO, GENDER, and AGE** ($F=6.55$, $p=0.011$) for MS_{FSc}. However, due to violation of assumptions (see 3.4.3.3.), one has to be cautious when interpreting this interaction.The significant interaction with AGE has already been shown above for MS_{FSc}.

Due to violation of assumptions, the interaction between **AGE, POR, and PHOTO** ($F=8.75$, $p=0.003$) slightly fails to fulfill the stringent conditions (3.4.3.3.). Again, caution is advised when interpreting the interaction of AGE, POR, and PHOTO. Although p-value almost reaches stringent criteria, F-value still is small compared to F-value of other interactions (GENDER-AGE and GENDER-PHOTO).

3.4.3.5. Conclusions

The effect of predictors is significant for those variables that substantially contributed to the regression model in chapter 3.4.2.3. Also, covariates, ØDOLE and BMI, are significant except BMI for MS_{FSc}. BMI showed a very weak and doubtful contribution to the regression model of MS_{FSc}. Although assumptions are violated, results from ANCOVA are consistent with outcomes from Multiple regression (of course, both methods are mathematically related).

Interactions between predictors are different between models of MS_{FSc} and $SLD_{Ø}$. None of the interacting effects between predictors is significant on the 0.01% level for $SLD_{Ø}$. Triple interactions (AGE-GENDER-PHOTO and AGE-POR-PHOTO) can be cautiously discussed for MS_{FSc}.

4. Discussion

The circadian clock controls daily life, from molecules to behaviour. By measuring gene expression or physiological parameters, the clock can be investigated in the laboratory. For large scale investigations of the human circadian clock, these methods are either not applicable or hardly feasable. The daily change of sleep and wakefulness is also controlled by the clock. Chronotype defines the position of sleep and wakefulness within the 24 hour day and can be easily assessed by a questionnaire. The MCTQ has been developed to quantitatively assess chronotype. The pilot study by Roenneberg *et al* (2003a) yielded convincing results on the distribution of chronotypes, with emphasis on the specific differences between work days and free days. A preliminary validation was performed by the authors using sleep log data of 30 university students (21.0 ± 2.5 years). An in depths validation with a larger, randomly chosen sample, however, was still waiting to be done. Validation was performed by Test-Retest-Reliability and a comparison of MCTQ and sleep log data (from the same individuals, respectively)

4.1. Validation of the MCTQ

4.1.1. MCTQ vs MCTQ (Test-Retest-Reliability)

Note: The list of abbreviations is available as fold-out on the last page

Retesting participants proves the MCTQ to give extremely stable estimates of phase of entrainment (chronotype) including all essential parameters. SE_F shows the weakest correlations, especially for Males and Age ≤30. Both sub-groups seem to have more difficulties in assessing their SE_F. Possibly, younger people, especially students, more often have varying schedules and no strict separation between work days and free days while older people could more often have stable employments and monday-to-friday work days. This could influence the accuracy of assessing SE_F. Males are on average later types than females and might therefore need recovery sleep more than females. Later chronotypes assess themselves slightly less accurate than early types, especially concerning SO_F. This is predictable because normal 9 to 5 work schedules usually do not harmonize with free day behaviour of late types (Roenneberg *et al*, 2003a). Still, all correlations are highly significant for MS_F and all essential parameters for the calculation of MS_{FSc} and $SLD_{ø}$ ($SO_{W/F}$, $SE_{W/F}$). There are no significant differences between dates of testing and retesting. Only ØDOLE is significantly different (paired t-Test <0.001). Testing sub-groups shows only Females, Age >30 years, and MS_{FSc}<4.28 to be significantly different. This could be due to longer days in summer.

Additionally, 15 persons were tested within a relatively short period (3 weeks). Results again show highly significant correlations ($p<0.001$) for all parameters essential to determine MS_{FSc} (SO_W, SE_W, SO_F, and SE_F), except for SE_F. In contrast, SO_F shows the highest correlation ($r=0.919$) of all parameters. Paired t-Test shows no significant differences for all of these parameters ($p>0.112$). The non-significant correlation of SE_F could be explained by the fact that "free days" is mainly understood as days without working schedules. Comments from participants indicated that some people (especially freelancers or students) get confused about the term 'free days'. That could be put right by taking care of unambigious advice for handling the questions. The high corralation of SO_F indicates that SO_F is very stable, in contrast to SE_F. This strengthens the correction of MS_{FSc} which assumes that most people recover (due to an accumulated sleep need) by sleeping in and not by going to bed earlier. For both, 6 months and 3 weeks retesting, MS_F correlates with high significance.

4.1.2. MCTQ vs Sleep log

The MCTQ has been compared with the MEQ score using a sample of about 2500 university students (Zavada *et al*, 2005). The authors showed that MS_F significantly correlates with the MEQ score ($r=-0.73$). Other parameters, like $SO_{W/F}$, $SE_{W/F}$, or any other point of sleep phase, show weaker correlations. These findings support the results shown in chapter 3.2.2. that MS_F is the best reference point to reflect phase of entrainment.

In constant routine protocols, dim light melatonin onset (DLMO) is commonly used as reference point to assess the phase of the endogenous pacemaker (Lewy *et al* 1995; Benloucif *et al*, 2005, Wright *et al*, 2005). Martin & Eastman (2002) compared SO_F, SE_F, and MS_F with the DLMO ($n=26$). For 92% of the subjects, DLMO, predicted by MS_F, was within a range of 1 hour and never exceeded 1.5 hours and DLMO showed highest correlation with MS_F ($r=0.89$). However, DLMO correlated significantly but weaker with the MEQ score ($r=0.49$). The authors recommend using sleep logs for the assessment of circadian phase, even when sleep schedules are irregular. Sleep logs thus seem to give a good reflection of average sleeping behaviour.

The MCTQ was validated with 6 week long sleep logs. Work days and free days were analyzed separately and MS_F reflected the averaged mid-sleep of all indicated free days. $MS_{F\text{-}MCTQ}$ and $MS_{F\text{-}Sleep\ log}$ correlate with high significance. There is a tendency of overestimation of MS_F towards extreme chronotypes. The earlier or later the MCTQ derived MS_F, the more, on average, there is a difference between $MS_{F\text{-}MCTQ}$ and $MS_{F\text{-}Sleep\ log}$. To face the problem of uneven numbers of subjects along the distribution of MS_{FSc} (more late and early types than 'normal types' (see chapter 3.2.2.) which leads to higher weighting of the ends of the distribution, sub-group analyses were performed. Regression slopes are very similar for all groups

analysed (MS_{FSc}: <2.17 ; 2.17<4.28 ; 4.28≤7.25 ; >7.25). Average chronotypes (MS_{FSc} 4-5) are most precise in assessing actual sleep times with the MCTQ. Differences between assessed sleep times and logged sleep times increase almost linearly towards both ends of the distribution. Values of $MS_{F\text{-}MCTQ}$ and $MS_{F\text{-}Sleep\ log}$ are not significantly different the total sample (paired t-Test: p=0.735) and the 'normal' group (MS_{FSc} 2.17≤7.25, paired t-Test: 0.688) indicating the precision of the 'normal' chronotypes but also of the total sample. Paired t-Test also shows MS_F to correspond best between MCTQ and sleep log compared to SO_F and SE_F.

Different reasons for the tendency of overestimation might apply to early types and late types. Both groups are negatively affected by different kinds of schedules. It is hardly bearable for extreme late types to adjust to 'normal' working schedules (e.g. 9 to 5). On the other hand, social life often starts in the evening when many early types think of falling asleep. Most other people are, for the respective group, earlier or later chronotypes, respectively. Feeling extreme could make an extreme type overestimate his or her actual sleep times. Variation in sleep times on free days (3.2.3.1.) and differences between sleep times on work days and on free days (3.2.3.2.) also have an influence on the ability to assess average sleep times with a single value (as it is the case in the MCTQ). The more regular sleep times, both within free days and between work days and free days, the more precise the assessment via MCTQ.

Deviations between sleep times derived by the MCTQ and by sleep logs are very systematic. Participants, especially when tested for genetic analysis, should receive more detailed information prior to the MCTQ in order to prevent misinterpretations of the own circadian rhythm. Another way could be to correct MCTQ derived MSF by the deviation between $MS_{F\text{-}MCTQ}$ and $MS_{F\text{-}Sleep\ log}$. Both possibilities, however, have to be tested with a new set of data. Nevertheless, the MCTQ is capable of assessing actual chronotype by using sleep times. There is no significant difference between MCTQ and sleep log for SE_W for no chronotype group (**Table 3.3.**). Conventional work times are strict schedules for most chronotypes and usually very constant. This indicates that assessment of sleep times is precise when actual sleep times are more or less constant.

4.2. Complexity of the MCTQ

The MCTQ has been developed with the attempt to quantify the phase of the human sleep-wake cycle. Sleep times ($SO_{W/F}$, $SE_{W/F}$, and $MS_{W/F}$) assessed with the MCTQ have been shown to reliably represent actual sleep times (see chapter 3.2). In the pilot study of Roenneberg *et al* (2003a), only $SO_{W/F}$ and $SE_{W/F}$ were used to calculate MS_W, MS_F, SLD_W, and

SLD_F. The MCTQ actually provides more questions ($BT_{W/F}$, $FA_{W/F}$, $IWT_{W/F}$,and $DIP_{W/F}$). Two additional questions are asked for free days ('My dream would be to sleep until…' and 'If I get back to sleep, I sleep for another…').

Roenneberg *et al* (2004a) found that $SLD_{ø}$ is a characteristic of sleep independent of its timing (MS_{FSc}). It was, therefore, investigated if there are other independent characteristics within the set of MCTQ questions or to reveal redundancies. All MCTQ variables (questions) highly correlate with MS_{FSc} except for DIP_W and DIP_F. Residuals of regression analyses of MS_{FSc} with all other time points are normally distributed, indicating that there is no clear and distinguishable group (e.g., showing very long or short times to wake up or to fall asleep).

Using Factor Analysis (Principle Component Analysis, PCA), a common statistical method to reduce the complexity of datasets, independent structures, representing groups of highly correlating variables, can be revealed. Indeed, PCA showed that MS_{FSc} and $SLD_{ø}$ are highly independent of each other. Also, as suggested in chapters 3.3.1.-3., DIP_W and DIP_F form a highly independent structure within the set of MCTQ questions.

In addition $BT_{W/F}$, $SO_{W/F}$, $MS_{W/F}$, $SE_{W/F}$, $IWT_{W/}F$, $FA_{W/F}$, and $DIP_{W/F}$, three parameters derived from MCTQ questions were included in PCA, namely SLD_W, SLD_F and $SLD_{ø}$. Three different Factors (structures underlying a set of variables) could be determined. Factor 1, *'Chrono-type'*, representing phase of entrainment (MS_{FSc}), explains more than half of the total variance assessed by the MCTQ. The term 'total variance' relies on the variance provided by all questions, thus, when removing questions, more or less information gets lost. Because most parameters highly correlate with MS_{FSc} (all exept for $SLD_{W/F/ø}$ and $DIP_{W/F}$), they only contribute little to the total variance and are redundent. The Factor 2, *'Duration'*, could also be called α/ρ because it describes the ratio of rest (ρ) and activity (α). About a fifth of total variance (18.6%) can be explained by *'Duration'* (or α/ρ). The Factor 3, *'Social conventions'*, consists only of $DIP_{W/F}$ with practically no contribution of any other parameters and explains less than a tenth of total variance (6.6%).

Although being independent of other questions, DIP_F shows a relatively high association with Factor 1 (**Table 3.14.+3.15.**) while DIP_W doesn't. Many people complain about feeling tired after meals (results from an unpublished questionnaire about daily behaviour, Appendix1, Fig.2). On work days, for most people lunch-hour depends on a strict schedule and food intake is restricted to a fixed time. On free days, meal times are less restricted but could still be under control of some social constraints. Thus, if there were no restrictions on meal times, DIP would rather fall into Factor 1. Factor 4, therefore, does not seem to reflect an independent endogenous characteristic like phase (*'Chronotype'*) or α/ρ (*'Duration'*) but is a result of independent, exogenous influences.

None of the variables (questions) shows its strongest association with Factor 3 (**Table 3.14.+3.15.)**. SE_W shows the highest and SO_F the weakest association with Factor 3. This

indicates that SO_F is the parameter that is least affected by work day schedules while SE_F actually is affected by work day schedules. Especially for late chronotypes, work day constraints can result in what might be called 'social jetlag' (Wittmann *et al*, 2006), shifting their sleep/wake behaviour for hours every weekend (for examples see **Fig.3.14.A+C**).

For two reasons the structure of Factor 3 also strengthens MS_{FSc} to be a sensible correction of MS_F and a reliable marker for phase of entrainment: 1. MS_{FSc} does not substantially load on Factor 3 (and also not on Factor 4), indicating an independence of social and work day constraints. 2. As described in chapter 2.4.2., MS_{FSc} is a correction for additional sleep on free days (due to sleep dept on workdays) assuming a constant sleep onset. $SLD_{Ø}$ also does not load substantially on Factors 3 and 4 and could be a good estimate of average sleep need.

The structure of the factor solution is identical when separately analysing Females, Males, Age ≤30, and Age >30. Most interestingly, Age ≤30 is more affected by *'Social jet-lag'* (13.6% vs 9.5%) and slightly less affected by *'Social conventions'* (6.3% vs 7.1%). This could be explained by an, on average, more stable employment of people >30 years which leads to a more constant influence of work times and a greater importance of social life, e.g. shared meal times or any other familiy business.

4.3. Factors associated with chronotype

4.3.1. Statistical models

The circadian clock is a self-sustained endogenous pacemaker regulated by the interaction of many known and more unknown genes (Roenneberg & Merrow, 2003). However, the clock is not a strict machine but is highly adaptive to entraining signals (chapter 1.3.; Roenneberg *et al*, 2003b; Duffy & Wright, 2005). When asking people for their sleep-wake behaviour (as the MCTQ does), one usually asks for an entrained rhythm under normal life conditions. It is, therefore, crucial to identify and quantify potential biological (or endogenous) and social (or environmental) influences that intra- and inter-individually have the potential to alter the phase of an entrained sleep-wake rhythm.

From previous results of this study it is hypothesized that chronotype (phase of entrainment, MS_{FSc}) is independent of the ratio of sleep and wakefulness (α/ρ, $SLD_{Ø}$). For both, an association with AGE, GENDER, ØDOLE, PHOTO, LAT, POR, and BMI was estimated using Multiple regression models. It has to be mentioned first that regression models do not fulfill all statistical assumptions (see chapter 3.4.2.2.). While deviations from normal distribution of quantitative variables did not substantially change outcomes, the non-linear change of MS_{FSc}

with increasing age is a reason for concern. Especially the impact of the variable AGE can differ between conditions (**Table 3.16.**), ranging from 3.8% to 4.9% of total explained variance of MS_{FSc}. However, in this part of the analysis, it is not of prime interest to predict MS_{FSc} or $SLD_{Ø}$ using other variables but to I) identify potential influences and II) assess the impact of each influence on chronotype compared to all other influences included in the models. More interesting than absolute values of variance explained by potential influences is the ratio of explained variances and differences between Gender and Age groups.

No transformation has finally been applied because it didn't improve the models in terms of normal distributions (Kolmogorov-Smirnov z-values still indicated a significant difference from normal distribution, **Table 3.16a**). Except for strong modifications (only Age >20 + $MS_{FSc\ log10}$ + AGE_{log10}), the order of variables, ranged by r^2, remains the same (**Table 3.16b**). An even weaker effect of log-transformations or of a restriction to Age >20 is apparent for $SLD_{Ø}$.

The first obvious observation is that MS_{FSc} and $SLD_{Ø}$ are differently associated with potential influences. For the total sample population, AGE and GENDER show about the same association with MS_{FSc} and $SLD_{Ø}$. LAT has been removed in both models, presumably because the range of latitude was too low. Longitude might have been a better parameter to test. Within Germany, time of dawn and dusk can differ by 36 min (between Aachen and Görlitz as western and eastern end, respectively). Depending on postal codes, longitude could be coded as continous variable to quantify a potential variability of MS_{FSc} and $SLD_{Ø}$ due to a difference in the relation of sunrise and sunset to actual time. ØDOLE, PHOTO, POR, and BMI, in contrast, are very differently associated with MS_{FSc} and $SLD_{Ø}$. Furthermore, there are differences between Work days & Free days, Females & Males, and Age ≤30 & Age >30 which will be discussed in detail.

4.3.2. AGE

A strong impact of AGE on MS_F and SLD_F and the weak impact of AGE on MS_W and SLD_W is not surprising. The great difference between work days and free days reflects sleep dept and recovery sleep (which leads to a delayed phase). Chronotype changes with age (Roenneberg *et al*, 2004b; Dijk *et al*, 2000) and the impact of age is supressed on work days due to work schedules. On free days, the impact of age is the sum of actual chronotype and sleep dept because sleep dept depends on chronotype (Roenneberg *et al*, 2003a) and chronotype depends on age.

Sleep duration becomes shorter with increasing age (**Fig.3.23.**; Dijk *et al*, 2000) and, for most people, is restricted on work days, especially for later chronotypes (Roenneberg *et al*, 2003a). The different impact of AGE in Work day models and in Free day models again reflects sleep dept and sleep recovery.

The change of chronotype throughout life is different for females and males (Roenneberg *et al*, 2004b; Fig.1d, Appendix 1). Between 15 and 50 years of age, males are on average later types (have a later MS_{FSc}) than females. The maximum difference is reached at about the age of 20 years, then decreasing constantly until fading out at about the age of 50. The range of average MS_{FSc} throughout life is greater for males, thus age explains a higher amount of variance of MS_{FSc} for males.

For both, phase of entrainment and sleep duration, results support prior observations on that issue (Roenneberg *et al*, 2003a; Roenneberg *et al*, 2004b).

4.3.3. GENDER

Females show an advanced MS_{FSc} and a longer $SLD_{ø}$ compared to Males. The strength of association of GENDER with MS_F and MS_W is almost similar (**Fig.3.25.**). SLD_F is very weakly associated with GENDER while the association with SLD_W is relatively high. Thus, Females appear less sleep deprived on work days possibly because of an earlier sleep phase. Females can sleep earlier and reach a longer sleep duration before the alarm clock wakes them on work days.

The average difference of MS_{FSc} between Males and Females is higher for Age ≤ 30 than for Ages >30 (see **4.3.2.**; Roenneberg *et al*, 2004b; Fig.1d, Appendix 1). The association of GENDER and MS_{FSc} is, therefore, higher for the younger group (**Fig.3.4.5.**). This difference is less pronounced for $SLD_{ø}$. The change of $SLD_{ø}$ throughout life seems to be more similar between females and males than the change of MS_{FSc}.

4.3.4. Average daily outside light exposure (ØDOLE), Photoperiod (PHOTO), and Latitude (LAT)

MS_{FSc} and $SLD_{ø}$ are very differently associated with social influences. The significant but very weak association between $SLD_{ø}$ and LAT (only for Age >30) is very likely due to the high number of cases (n=8692, **Table 3.19.**) and will not be included in further interpretations. In general, $SLD_{ø}$ is less associated with changing light conditions as it is MS_{FSc}. Big differences between Gender and Age groups can be seen for MS_{FSc} while they are small or even not apparent for $SLD_{ø}$. Increasing ØDOLE is much more linked to an advanced MS_{FSc} for Males and Age >30. Females are almost not associated with PHOTO while for Males, the association is twice as high as for the total sample.

Shorter PHOTO (winter months, Oct-Mar) is also more linked to advanced MS_{FSc} for Age >30 (**Fig.3.26.**, **Table 3.19.**). This could indictate that males and older people are more sensitive to light effects. Older people are reported to have lower amplitudes of circadian

rhythms, e.g. body temperature (Dijk *et al*, 2000; Monk, 2005). A generally lower amplitude of the circadian pacemaker could result in higher responsivness to light. The decrease in amplitude has also been shown to be greater for males (Moe *et al*, 1991; Campbell *et al*, 1989). A general advance of circadian output rhythms has been described (Czeisler *et al*, 1992; Duffy *et al*, 2002; Monk *et al*, 1993). In case the second, unknown, pacemaker driving the sleep-wake cycle (Kronauer *et al*, 1982; Daan *et al*, 1984) does not change its periodicity with age, the lag between circadian phase and sleep wake cycle would be larger (Dijk *et al*, 2000). Awakening could thus appear at a phase of the circadian pacemaker more responsive to light and might lead to a even stronger phase advance.

The differences between Females and Males are more difficult to interpret. The visual system of males could be more effective in transmitting light information to the SCN, maybe due to a higher number of melanopsin containing retinal ganglion cells. Also, the amplitude of circadian rhythms could be lower for males of all ages, not only for older males. A methodolocical reason could be that females and males differ in the ability to assess the time spent outside. It is, however, not deducable which group does this more accurately. In contrast to ØDOLE, the time of filling out the MCTQ can be exactly determined. Shorter PHOTO is much more linked to delayed MS_{FSc} in males. This offers room for some wild and theoretical speculations: Maybe, in early evolution of mankind, the circadian system of males had to be more adaptive to changing light conditions (day-night, summer-winter) to be succesful hunter-gatherers while females mainly had to care for the offsprings. In contrast, the female circadian clock could be more sensitive to social entraining factors and social synchronization has been shown for females (McClintock, 1971). The fact that males, on average, have a later sleep phase could also result from the division of labour. For females, it could have been of advantage to be early in order to meet the demands of social life (e.g. offsprings) while the ability of males to stay awake longer could have provided more opportunities for hunting (e.g. nightactive prey).

Interestingly, ØDOLE is more associated with MS_W than with MS_F. However, these results are most likely to be due to other influences. MS_W is advanced rather by early work schedules. The effect of ØDOLE on MS_F is smaller than on MS_{FSc} (**Table 3.17 & Table 3.18**). The average delay of MS_F caused by recovery sleep on free days could counteract to an advancing effect of light. Taking this into account, the association of ØDOLE with MS_{FSc} could reflect the possible effect of light on the sleep-wake rhythm better than ØDOLE with MS_F.

The MCTQ asks for ØDOLE separately for work days and for free days. Although light has an effect on the human circadian clock throughout the whole day (no dead zones as shown in **Fig.1.3.**A; Jewett *et al*, 1997), the clock is most sensitive to light during early subjective night (causing phase delays) and early subjective morning (causing phase advance, see chapter 1.3.2.2.). It could be more informative to ask for ØDOLE separately e.g. for morning,

afternoon, and evening or to offer the opportunity to indicate the duration of outside light exposure together with a concrete time. However, this might be to demanding for many people. Still, an assessment of outside light exposure for at least the first and the second half of the day is applicable.

4.3.5. Place of residence (POR)

The fact of living in a city with more than 100,000 residents or in a smaller city, town, village, or even a solitary farm is associated only with MS_{FSc}. People from big cities, on average, are later chronotypes. For the total sample population, POR is even stronger associated with MS_{FSc} than it is ØDOLE. However, the association with ØDOLE can be assessed more precisely (see text above). No differences are apparent between Gender and Age groups. POR is similarely associated with MS_{FSc} for Females & Males and Age ≤30 & Age >30 (**Fig. 3.26.**); the association is much stronger with MS_W than with MS_F (**Fig. 3.25.**).

Life in bigger cities may be more stressful than in smaller cities or in the countryside: Everyday traveling with public transport (bus, suburban train) or car (traffic jams), crowded places and streets,… . Stress is known to be a cause of sleep disturbancies (Hohagen, 1999). City people might take longer to calm down in the evening due to elevated stress during the day and this could lead to a delayed sleep phase. One could think that this also leads to shorter sleep duration, at least on work days, because of work schedules. Interestingly, neither SLD_W, nor SLD_F are substantially associated with POR. Maybe, flexible work times are more common in urban agglomerations and sleep is less restricted by the work day clock. An additional factor for delayed sleep could be that there is more program of cultural events and a nightlife in big cities (cinemas, discos, pubs). People might feel more invited to go out in the evening for social contacts.

It is easy to shield ones home from ambient artificial outside light in the evening, so this is rather unlikely to be the cause of a delayed sleep phase in big cities. Also, there are no differences between Gender and Age groups as it is the case for ØDOLE and PHOTO. However, depending on the location, living in cities can be noisy in the evening, even when windows are closed, and noise can lead to stress.

There are numerous potential factors in a big city that could lead to a later sleep phase. To dissect POR into its components, additional questions are necessary, e.g. if people generally feel stressed by everydays life. The size of POR could be directly used in a quantitative scale (instead of the dichotomous scale < or > 100,000) to assess the kind of correlation (linear, exponential,…) between MS_{FSc} and POR.

4.3.6. Body mass index (BMI)

There is a mentionable but weak, negative association of BMI only with $SLD_{Ø}$. The association is about the same for Females and for Males but it is twice as high for Age >30 than for Age ≤30 (**Table 3.19.**, **Fig.3.26.D**). A relationship between increased BMI and short sleep duration has been shown by Taheri *et al* (2004). One possibility, discussed by the authors, could be that short sleepers open the fridge at unfavourable times due to later bed times. Also, hormons controlling appetite could be affected by short sleep duration. However, this study includes only obese people (BMI >30), so the statements made could be questionable. The results of this study show that the association of $SLD_{Ø}$ and BMI is age dependent. There is a positive and highly significant partial correlation between BMI and AGE ($r = 0.274$, $p<0.001$, $n=18424$; controlled for MS_{FSc}, $SLD_{Ø}$, and ØDOLE). There is no meaningful difference in the association between BMI & SLD_W and between BMI & SDL_F but interestingly, for both, SLD_W and SLD_F, the association is weaker than with $SLD_{Ø}$ (**Table 3.18.**, **Fig.3.25.**).

4.3.7. Interactions

The average chronotype has been identified to have a MS_{FSc} of 4.28 ± 1.36. Due to subgrouping by biological and social factors (**Fig.3.27.**), average values can differ between 3.77 ± 1.15 (Females ≥30, Apr-Sep, POR <100,000) and 5.42 ± 1.48 (Males <30, Oct-Mar, POR >100,000). Even when considering the limited explanatory power of the ANCOCA model, differences of average MS_{FSc} of more than 1.5 hours between sub populations indicate the enormous variability of chronotype due to biological and social factors (**Fig.3.28.A**). Interactions of factors (predictors) give rise to the assumption that females and males are differently influenced by certain factors and that the age dependent change of strength of influences is different between females and males (**Fig.3.28.C+E**).

The variability of $SLD_{Ø}$ between sub-populations is less pronounced (**Fig.3.28.D+F**). The average $SLD_{Ø}$ is 7.46 ± 1.00 and $SLD_{Ø}$ can vary from 7.14 ± 0.94 (Males, >30, Apr-Sep, POR <100,000) to 7.83 ± 0.98 (Females, ≤30, Oct-Mar, POR <100,000). There are significant differences between females & males and ≤30 & >30 (**Fig.3.28.A**). There is, however, no difference in the age-dependent decrease of $SLD_{Ø}$.

4.4. Conclusions & Recommendations

This study had two main objectives: First, the MCTQ, a recently developed questionnaire for the quantitative assessment of chronotype, was validated (3.2. & 4.1.) and its structure was optimized towards a maximum amount of information with a minimum number of questions (3.3. & 4.2.). Second, potential biological and social influences on the sleep-wake cycle were scrutenized in order to 'clean' chronotype from environmental bias (3.4. & 4.3.).

4.4.1. Improvement of the MCTQ

Questions revealed to be redundent by PCA can be removed from the questionnaire without losing much information. Only $SO_{W/F}$ and $SE_{W/F}$, are necessary to determine chronotype. A discrimination between BT and the time of switching off the light in order to fall asleep on one hand and SE and time of leaving bed on the other hand makes the MCTQ unambigeous, thus these questions are added. However, the new questions do not give more information but make clear that BT, switching off lights, and SO are different questions, as well as SE and getting out of bed.

The MCTQ also asks for subjective assessment of chronotype on a scale from 0 (extreme early type) to 6 (extreme late type). Unlike the distribution of MS_{FSc}, the distribution of subjective chronotype does not resemble a normal distribution. Most people assess themselves as moderate late types (28%), probably due to early work times which most people regard as too early (Roenneberg *et al*, 2004; Appendix1, Fig.3). Subjective chronotype rating thus does not reflect actual sleeping behaviour. In contrast, MS_F is a reliable estimate of actual sleep times assessed by sleep logs which in turn are reported to reliably represent DLMO (Martin & Eastman, 2002). Therefore, subjective chronotype rating will be not included in the improved version of the MCTQ.

$DIP_{W/F}$ could be a reference point for a decreased state of alertness during the day, controlled by the circadian and/or the homeostatic oscillator. As discussed before, $DIP_{W/F}$ is highly influenced by social schedules, e.g. meal times. It is doubtful if $DIP_{W/F}$ reflects an independent structure of chronotype or if it is rather an artefact of social constraints. Therefore, $DIP_{W/F}$ will not be considered in the improved version of the MCTQ.

For the calculation of MS_{FSc}, the general ratio of work days and free days of 5:2 was used. Asking for actual number of work days per week allows an individual weighting of work days and free days. The improved MCTQ asks for the number of work days per week.

After removement of redundant questions and the addition of three more questions, the MCTQ comprises less than one page (Appendix 3) and can be filled out in 3 minutes. A reliable and quick assessment of chronotype can be used in numerous situations: in the hospi-

tal or at the general practitioner to improve medication or for employers to assess optimal work times for employees. Also, participants in the online chronotype survey could focus on the essential questions. Short questionnaires are always appreciated by people and could improve the reliability of results.

4.4.2. Properties of chronotype

Following the two-oscillator model (see chapter 1.5.5.2.), the change of sleep and wakefulness is regulated by a circadian oscillator and a homeostatic or hourglass oscillator. In this model, the circadian oscillator mainly determines the position of sleep phase within the 24 hour light-dark cycle while maintainance of sleep is likely to be an interplay of both oscillators (Dijk & von Schantz, 2005; Dijk *et al*, 2000). Phase of entrainment and α/ρ (sleep duration) can be determined with the MCTQ. Phase could describe the behavioural output of the circadian oscillator, α/ρ the output of the homeostatic or both oscillators. On the population level, both properties are statistically independent of each other.

The term chronotype referred to phase of entrainment and not to sleep duration in the pilot study of Roenneberg *et al* (2003a). However, both contribute to the variation of the daily change of sleep and wakefulness which involves many brain areas (Pace-Schott & Hobson, 2002). The genetic basis of chronotype (phase of entrainment) has been described in humans and in non-human mammals (1.4.5.) and is assigned to the SCN (1.4.3.). Also, there is evidence that sleep duration (α/ρ) is genetically controlled (Franken *et al*, 2001). Non-clock genes and clock genes could be involved in the manifestation of sleep duration (Tafti & Franken, 2002; Naylor *et al*, 2000). The two oscillator model from Pittendrigh and Daan (1976b; 1.4.6.2.) might explain the adaptability to changes in day length (Daan *et al*, 2001). Two distinct oscillators could also explain the achievement of sleep duration (α/ρ). Different phase angles of oscillators, respectively to dawn and dusk, could cause both sleep phase and sleep duration (**Fig. 4.1.**).

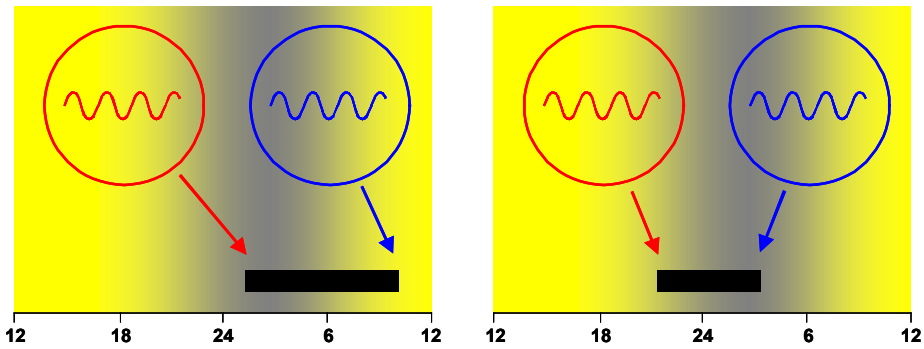


Fig. 4.1.: A model of two oscillators defining both sleep phase and sleep duration. Every oscillator has its own properties (e.g. tau, responsivness to entraining signals,…) locking differently to dawn (morning oscillator, blue) and dusk (evening oscillator, red). Black bars represent sleep.

Although an influence on sleep homeostasis has been shown in mice only for the *Clock* gene (Naylor *et al*, 2000) and not for components of the hypothesized morning and evening oscillator (Per1/2 and Cry1/2, Daan *et al*, 2001), the general framework of sleep duration could be assigned to or at least influenced by the circadian clock.

4.4.3. Future directions for a proper chronotyping

This study showed the potential influence of biological and social factors. However, only associations and not causalities were obtained. Results should, therefore, be used to formulate hypotheses and design specific experiments for single factors.

When considering the tendency of overestimation of extreme chronotypes, the MCTQ is a highly reliable instrument for the assessment of chronotype. In contrast to the Horne-Östberg MEQ, the quantitative measure of chronotype can be corrected for biological and social factors. The improved version of the MCTQ can be used to determine chronotype under normal life conditions in a first round. Depending on the purpose of further investigations, different chronotypes can be easily selected. Also, a quick an reliable assessment of chronotype is possible whereever it is needed (e.g. physicians, employers,…). Based on results of this study and further experiments on biological and social factors, one should be able to break down chronotype into genetic causes by quantifying as many non-genetic influences as possible. This can help to reduce false positive results and to improve the attempts at identification of new human clock genes.

5. Summary / Zusammenfassung

5.1. Summary

Like most other organisms, human behaviour is under control of a circadian (= about a day) clock which can adapt to the daily change of light and darkness. The capability of the circadian clock to synchronize to the light-dark cycle is highly systematic and under genetic control. Daily behaviour of humans can vary greatly, leading to different chronotypes and extreme chronotypes are commonly described as 'owls and larks'. While larks fall asleep in the early evening and wake up early in the morning, owls go to bed when larks wake up, sleeping until about noon. Recently, the Munich ChronoType Questionnaire (MCTQ) has been developed in order to quantitatively assess chronotype and to face problems arising from common work day schedules. Chronotypes are normally distributed in the general population. MS_{FSc}, the middle of sleep phase on free days corrected for sleep dept accumulated on work days, is used as reference point for chronotype. The objectives of this study were both to validate and improve the MCTQ and to identify and estimate biological and social factors that potentially influence the distribution of chronotypes.

Test-retest reliability was controlled at an interval of six months (n=101). Both dates show a highly significant correlation ($p<0.001$) and no significant difference (paired t-Test: $p>0.027$, Bonferoni corrected level of significance: $p=0.0012$). Test-retest reliability was controlled separately for females and males, different age (≤30 years and >30 years), and chronotype groups (MS_{FSc} ≤4.28 and >4.28). A sample of 15 people was tested at an interval of three weeks. The results of both dates correlate with high significance ($p<0.001$) and paired t-Test showed no significant difference between both dates ($p>0.05$, Bonferoni corrected level of significance: $p=0.002$).

The capability of the MCTQ to assess actual sleep times was tested with 6-week long sleep logs (n=628). Again, gender, age, and chronotype groups were evaluated separately and chronotypes were separated into five different groups (MS_{FSc} <2.17; 2.17≤7.25; 2.17<4.28; 4.28≤7.25; >7.25). Assessed (MCTQ) and actual (sleep log) sleep times correlate with high significance ($p<0.0001$, Bonferoni corrected level of significance: $p=0.0007$). There is a tendency of overestimation of chronotype towards extreme chronotypes. While average chronotypes (MS_{FSc} 4-5) can assess their actual sleep times very precisely, earlier chronotypes (MS_{FSc} <2) assess themselves earlier and late chronotypes (MS_{FSc} >7) assess themselves later as they actually are. Due to this overestimation, paired t-Test showed assessed and actual sleep times to be significantly different in most of the sub-groups ($p<0.0001$, Bonferoni corrected level of significance: $p=0.0007$). However, the tendency of overestimation is highly systematic and a linear correction could be applied for further investigations.

Principal Component Analysis (PCA), containing all questions of the MCTQ that could represent a phase reference point of daily behaviour, resulted in a four factor solution and revealed several redundancies in the MCTQ. Besides phase of sleep, as represented by MS_{FSc}, duration of sleep, as represented by average sleep duration, was identified as independent property of the sleep-wake cycle. Phase of entrainment explaines about 50% of total variance and sleep duration about 20% of total variance within the set of MCTQ questions. Most questions, except mid-day dip, are highly associated with the same factor as MS_{FSc}, thus not representing statistically independent dimensions of daily behaviour. Another factor is represented only by mid-day dip and explains about 7% of total variance. However, the independency of this factor might rather result from social influences. The last factor (about 12% of total variance) is dominated by sleep end on work days which limits sleep for most people on work days. This factor does not represent an independent structure of the sleep-wake cycle but the influence of the social clock on daily life.

Using Multiple regression analysis, the influence of biological and social influences on chronotype has been quantified in relation to each other. Phase and duration of sleep are differently associated with age, gender/sex, daily outside light exposure, photoperiod (season), BMI, and Place of residence. Phase of entrainment is substantially associated with age, gender/sex, daily outside light exposure, photoperiod (season), and Place of residence, while sleep duration is associated only with age, gender/sex, daily outside light exposure, and body mass index (BMI). Analysis of covariance (ANCOVA) showed interacting effects between gender/sex & age and gender/sex & photoperiod (season) only for sleep phase.

As a results of this work, the Munich ChronoType Questionnaire (MCTQ) has been proven to be a reliable, quantitative tool for the assessment of chronotype. Its precision, shortness, and the ability to correct for factors influencing chronotype make it the most preferable questionnaire in human chronoscience. In depth statistical analysis with extreme high numbers (up to n=29,000) hypothesizes that sleep duration is an independent property of daily behaviour besides chronotype, and showed both, chronotype and sleep duration, to be differently influenced by biological and social factors. This offers a good basis for new hypotheses and experiments.

5.2. Zusammenfassung

Wie die meisten Tiere und Pflanzen besitzt der Mensch eine circadiane Uhr. Diese sogenannte innere Uhr kann sich an den Wechsel von Tag und Nacht anpassen. Die Eigenschaften der inneren Uhr folgen systematischen Gesetzmäßigkeiten, sind genetisch bedingt und bestimmen unter anderem den sogenannte Chronotyp. Extreme Chronotypen werden auch 'Eulen und Lerchen' genannt. Während Lerchen am frühen Abend zu Bett gehen und am frühen Morgen von allein wach werden, gehen Eulen ins Bett wenn Lerchen bereits wieder aufstehen und schlafen dafür bis nach Mittag. Der vor einiger Zeit eingeführte Münchener Chronotyp Fragebogen (MCTQ) erlaubt das quantitative Erfassen des Chronotyps und berücksichtigt hierbei Unterschiede zwischen Arbeitstagen und freien Tagen. Der Chronotyp wird durch MS_{FSc} repräsentiert. MS_{FSc} bezeichnet die Schlafmitte an freien Tagen, welche um die verzerrenden Einflüsse von Schlafmangel an Arbeitstagen korrigiert wird. In der Bevölkerung folgt der Chronotyp annähernd einer gaußschen Normalverteilung. Das Ziel dieser Arbeit war die Validierung des MCTQ und das Aufdecken und Quantifizieren von biologischen und sozialen Einflüssen auf den Chronotyp.

Die Retest-Reliabilität des MCTQ wurde in einem Intervall von sechs Monaten getestet (n=101). Beide Zeitpunkte korrelieren hoch signifikant ($p<0.001$). Unterschiede zwischen beiden Zeitpunkten sind nicht zu beobachten (gepaarter t-Test: $p>0.027$, Signifikanzniveau nach Bonferoni Korrektur: $p=0.0012$). Die Retest-Reliabilität wurde für Frauen und Männer, ältere (>30 Jahre) und jüngere (≤30 Jahre) Teilnehmer und Chronotypgruppen (MS_{FSc} ≤4.28 und >4.28) getrennt durchgeführt. Eine Gruppe von 15 Personen hat den Fragebogen zweimal innerhalb von drei Wochen ausgefüllt. Die Ergebnisse korrelieren ebenfalls höchst signifikant ($p<0.001$) und es gibt keine signifikanten Unterschiede zwischen den Zeitpunkten des Ausfüllens (gepaarter t-Test: $p>0.05$, Signifikanzniveau nach Bonferoni Korrektur: $p=0.002$).

Die Möglichkeit, mittels des MCTQ die tatsächlichen Schlaf- und Wachzeiten einzuschätzen, wurde anhand von Schlaftagebüchern über einen Zeitraum von sechs Wochen überprüft (n=628). Es wurden wiederum Altersgruppen und Geschlechter getrennt getestet, die Teilnehmer wurden in fünf verschiedene Chronotypgruppen unterteilt (MS_{FSc} <2.17; 2.17≤7.25; 2.17<4.28; 4.28≤7.25; >7.25). Geschätzte und tatsächliche Schlafzeiten korrelieren hoch signifikant ($p<0.0001$, Signifikanzniveau nach Bonferoni Korrektur: $p=0.0007$). Extreme Chronotypen neigen dazu, ihren Chronotyp extremer einzuschätzen als er tatsächlich ist. Während durchschnittliche Chronotypen (MS_{FSc} 4-5) ihre tatsächlichen Schlafzeiten sehr präzise einschätzen können, über- bzw unterschätzen sich Spättypen (MS_{FSc} >7) bzw Frühtypen (MS_{FSc} <2). Daher sind bei den meisten Chronotypgruppen geschätzte und tatsächliche Schlafzeiten signifikant verschieden (gepaarter t-Test: $p<0.0001$, Signifikanzniveau

nach Bonferoni Korrektur: p=0.0007). Der Hang zur Überschätzung ist jedoch sehr systematisch (linear) und kann daher für weitere Untersuchungen korrigiert werden.

Mittels einer Haupkomponentenanalyse (PCA), unter Einbezug aller MCTQ Fragen, konnten vier unabhängige Faktoren identifiziert werden. Neben der Lage des Schlafes innerhalb des 24 Stunden Tages (MS_{FSc}) konnte die Dauer des Schlafes (durchschnittliche Schlafdauer) als unabhängige Eigenschaft des Tagesrhythmus identifiziert werden. Die Schlafphase erklärt hierbei ungefähr 50% der gesamten Varianz aller einbezogenen Fragen, Schlafdauer erklärt ca. 20% der gesamten Varianz. Die meisten Fragen zeigen eine starke Assoziation mit demselben Faktor wie MS_{FSc} und stellen daher keine unabhängige Eigenschaft des Tagesrhythmus dar. Ein weiterer Faktor wird lediglich von der Frage zum Mittagstief bestimmt und erklärt ca. 7% der gesamten Varianz. Die Unabhängigkeit dieses Faktors ist jedoch sehr wahrscheinlich äußeren, sozialen Einflüssen zuzuschreiben. Der letzte Faktor wird von der Frage nach dem Schlafende an Arbeitstagen bestimmt und erklärt ca. 12% der gesamten Varianz. Dieser Faktor stellt keine unabhängige Eigenschaft des Tagesrhythmus dar sondern beschreibt den Einfluss der sozialen Uhr (z.B. des Arbeitsbeginns).

Durch multiple Regression wurden der Zusammenhang von biologischen und sozialen Einflüssen mit dem Chronotyp und der Schlafdauer im Verhältnis zueinander bestimmt und quantifiziert. Schlafphase und Schlafdauer sind unterschiedlich mit Alter, Geschlecht, der täglichen Dauer des Aufenthalts im Sonnenlicht, Tageslänge (Jahreszeit), Body mass index (BMI) und Wohnort assoziiert. Schlafphase kann mit Alter, Geschlecht, der täglichen Dauer des Aufenthalts im Sonnenlicht, Tageslänge (Jahreszeit) und Wohnort maßgeblich in Verbindung gebracht werden während Schlafdauer nur mit Alter, Geschlecht, der täglichen Dauer des Aufenthalts im Sonnenlicht und BMI stark assoziiert ist. Ein zusammenhängender Effekt von Geschlecht & Alter und Geschlecht & Tageslänge konnte mittels Covarianzanalyse (ANCOVA) nur für die Schlafphase aber nicht für die Schlafdauer festgestellt werden.

Diese Arbeit zeigt, dass der Münchener Chronotyp Fragebogen (MCTQ) ein verlässliches, quantitatives Instrument zur Bestimmung des Chronotyps ist. Er ist schnell auszufüllen, die erhaltenen Angaben sind präzise, und er erlaubt die Korrektur des Chronotyps bezüglich biologischer und sozialer Einflüsse. Fundierte statistische Analysen mit einer sehr hohen Zahl an Fällen (bis zu n=29000) lassen darauf schließen, dass neben dem Chronotyp die Schlafdauer eine weitere, unabhängige Eigenschaft des Tagesrhythmus ist, und dass Chronotyp und Schlafdauer unterschiedlich mit biologischen und sozialen Einflüssen assoziiert sind.

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7. Erklärung

Hiermit erkläre ich, dass ich die vorliegende Arbeit selbsständigt angefertigt und nur die angegebenen Hilfsmittel und Quellen verwendet habe.
Ich habe bisher keinen Versuch unternommen, diese oder eine andere Dissertation, auch nicht in Teilen, einer anderen Prüfungskommision vorzulegen, noch habe ich mich erfolglos einer Doktorprüfung unterzogen.

München, den 13.04.06 

(Tim Kühnle)

8. Curriculum vitae

Name	Kühnle
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08/1995 – 09/1996	Rettungsdienst beim Deutschen Roten Kreuz, Kreisverband Schwäbisch Hall – Crailsheim e.V.
10/1996 – 08/2001	Studium der Biologie an der Bayerischen Julius-Maximilians-Universität Würzburg
12/2001 – 04/2002	Wissenschaftlicher Angestellter an der Medizinischen Poliklinik, Abteilung Klinische Biochemie, Ludwig-Maximilians-Universität München
01/2003 – 09/2005	Wissenschaftlicher Angestellter am Institut für Medizinische Psychologie, Zentrum für Chronobiologie, Ludwig-Maximilians-Universität München

Publikationen:

Chronoecology from Fungi to Humans
Roenneberg T, Tan Y, Dragovic Z, Ricken J, **Kuehnle T** und Merrow M
Buchkapitel, Sapporo Symposium 2004, S. 73-90

A marker for the end of adolescence
Roenneberg T, **Kuehnle T**, Pramstaller PP, Ricken J, Havel M, Guth A und Merrow M
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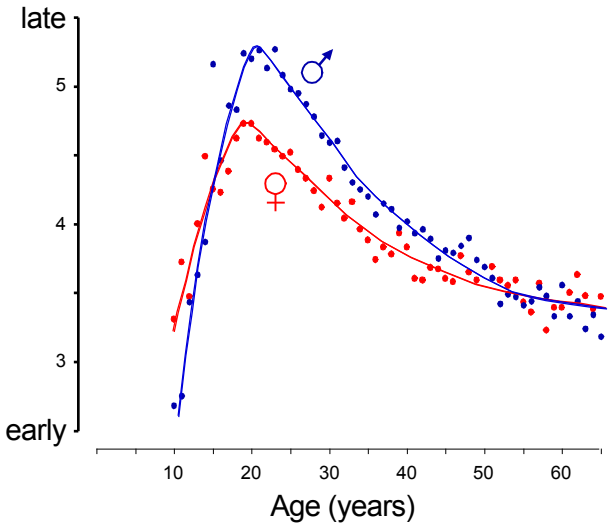
Appendix 1: Figures

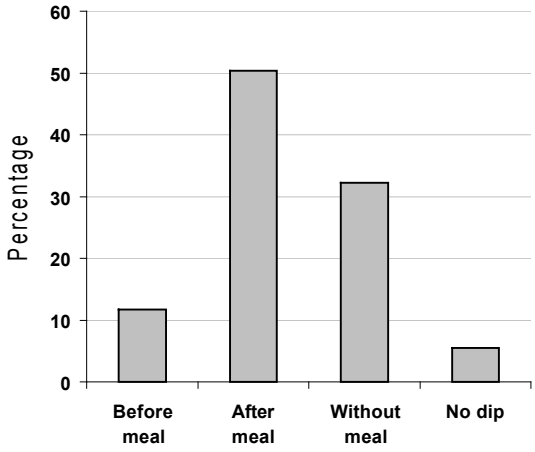
Fig.1



from Roenneberg *et al* 2004b

Fig.1d

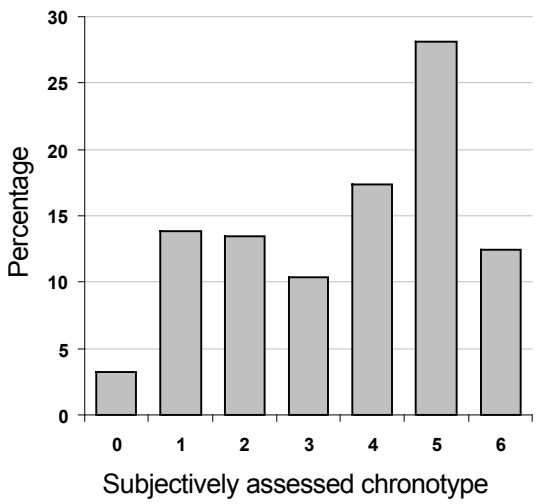
Fig.2



If you have an energy dip, is it related to food intake?

I have a dip before meals: 11.7 %
I have a dip after meals: 50.4 %
I have a dip without meals: 32.3 %
I have no dip: 5.6 %
(n=486, from an unpublished questionnaire containing questions about daily behaviour)

Fig.3



Distribution of subjectively rated chronotype

Chronotype		%
Extreme eary	(0)	3.3
Moderate early	(1)	13.8
Slight early	(2)	13.5
Normal	(3)	10.4
Slight late	(4)	17.4
Moderate late	(5)	28.1
Extreme late	(6)	12.4

(n=20781)

Appendix 2: Munich ChronoType Questionnaire (MCTQ)

MCTQ Page1

Personal information

People arrange their daily lives differently according to their "chronotype", e.g., when they go to bed and wake up. Our research tries to find the biological mechanisms behind the phenomenon of different "chronotypes". This page asks for personal information which is important for our investigations. Information about your age, gender, etc. are essential for all of our analyses. We ask for your name and address in case we wish to contact you, specifically with the possibility of collecting chronotype information from your extended family.
Please be assured that all of your personal information will be treated with the utmost confidentiality. This first page will be separated from the rest of the questionnaire. Upon arrival, your questionnaire will be encoded with a number that is added to all pages. The front page will be stored separately. This will enable us to get in contact with you later if necessary. All evaluations will be performed only with the anonymous, encoded pages which follow.

Important note: Please answer the questions concerning your present situation (e.g. in summer I usually stay outside longer than in winter). For changing conditions (e.g. beginning of work) please choose the most frequent or appropriate choice.

Subjects under 18 must have permission from a parent/guardian

Date and signature of parent/guardian: _____

Age: ____ female ☐ male ☐ Height _____ Weight _____

Name _____

Address _____

_____

Telephone number _____

e-Mail _____

I received this questionnaire via _____

MCTQ Page2

On workdays (don't fill out if you are retired)…

I get up at … _____o'clock
I need … _____min to wake up
I regularly wake up … before the alarm ☐ with the alarm ☐
From … _____o'clock, I am fully awake
At around … _____o'clock, I have an energy dip
On nights before workdays, I go to bed at … _____o'clock …
… and it then takes me … _____min to fall asleep
If I get the chance, I like to take a siesta/nap ... correct ☐ not correct ☐
if "correct": I then sleep for …_____ min
if "not correct': because I would feel terrible afterwards ☐

On free days (please only judge normal free days, i.e., without parties etc.) …

My dream would be to sleep until … _____o'clock
I normally wake up at … _____o'clock
If I wake up at around the normal (workday) alarm time, I try to get back to sleep...
correct ☐ not correct ☐
if I get back to sleep, I sleep for another … _____min
I need … _____min, to wake up
From … _____o'clock, I am fully awake
At around … _____o'clock, I have an energy dip
On nights before free days, I go to bed at … _____o'clock …
… and it then takes me … _____min, to fall asleep
If I get the chance, I like to take a siesta/nap ... correct ☐ not correct ☐
if "correct": I then sleep for …_____ min
if "not correct": because I would feel terrible afterwards ☐

---

once I am in bed, I would like to read for …_____ min, …
… but generally fall asleep after no more than … _____ min.

I prefer to sleep in a completely dark room correct ☐ not correct ☐
I wake up more easily when morning light shines into my room correct ☐ not correct ☐

How long per day do you spend on average outside (really outside!!) exposed to day light?
On work days: ___ hrs. ___min. On free days: ___ hrs. ___min.

MCTQ Page3

Self assessment
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 type. If, however, you regularly wake up and 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 type. In the following questions, you should categorise yourself and your family members.

Please tick only one possibility!

Description of categories:

extreme early type	= 0
moderate early type	= 1
slight early type	= 2
normal type	= 3
slight late type	= 4
moderatelate type	= 5
extreme late type	= 6

I am …	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
as a child, I was …	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
as teenager, I was…	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐

In case you are older than 65: in the middle of my life, I was …

0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐

My parents are/were…

Mother …	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
Father …	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐

My siblings are/were … (please underline Brother or Sister)

Brother/Sister	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
Brother/Sister	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
Brother/Sister	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
Brother/Sister	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
Brother/Sister	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
Brother/Sister	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
Brother/Sister	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐

My partner (girl/boy friend, spouse, significant other) is/was …

0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐

Appendix 3: MCTQ – Improved version

MCTQ Page 1 remains identical

MCTQ Page 2

Please give today's date: _____

Information about work days: I work _____ days per week

in case you don't work, please only fill out the information for free days

Before work days, I go to bed at _____ o'clock

… at _____ o'clock, I decide to sleep (switch off the light)

I need _____ minutes to fall asleep

On work days, I wake up at _____ o'clock (before the alarm ☐ with the alarm ☐)

…after _____ minutes I get up

Information about free days:

please judge days without special circumstances (Parties etc.)

Before free days, I go to bed at _____ o'clock

… at _____ o'clock, I decide to sleep (switch off the light)

I need _____ minutes to fall asleep

On free days, I wake up at _____ o'clock (without alarm ☐ with alarm ☐)

…after _____ minutes I get up

How long per day do you spend on average outside exposed to day light (no a roof above)?

On work days: ___ h ___ min On free days: ___ h ___ min

MCTQ Page 3 cancelled

Appendix 4: Sleep log

Begin of documentation (date): (please start with the corresponding day)										
Week	**from to**	**in bed**	**fell asleep**	**woke up**	**Alarm yes**	**no**	**out of bed**	**Work day**	**Free day**	**Comments (e.g. naps,...)**
1	**Sun/Mon**							Mon	Mon	
	Mon/Tue							Tue	Tue	
	Tue/Wed							Wed	Wed	
	Wed/Thu							Thu	Thu	
	Thu/Fri							Fri	Fri	
	Fri/Sat							Sat	Sat	
	Sat/Sun							Sun	Sun	
2	**Sun/Mon**							Mon	Mon	
	Mon/Tue							Tue	Tue	
	Tue/Wed							Wed	Wed	
	Wed/Thu							Thu	Thu	
	Thu/Fri							Fri	Fri	
	Fri/Sat							Sat	Sat	
	Sat/Sun							Sun	Sun	
3	**Sun/Mon**							Mon	Mon	
	Mon/Tue							Tue	Tue	
	Tue/Wed							Wed	Wed	
	Wed/Thu							Thu	Thu	
	Thu/Fri							Fri	Fri	
	Fri/Sat							Sat	Sat	
	Sat/Sun							Sun	Sun	
4	**Sun/Mon**							Mon	Mon	
	Mon/Tue							Tue	Tue	
	Tue/Wed							Wed	Wed	
	Wed/Thu							Thu	Thu	
	Thu/Fri							Fri	Fri	
	Fri/Sat							Sat	Sat	
	Sat/Sun							Sun	Sun	
5	**Sun/Mon**							Mon	Mon	
	Mon/Tue							Tue	Tue	
	Tue/Wed							Wed	Wed	
	Wed/Thu							Thu	Thu	
	Thu/Fri							Fri	Fri	
	Fri/Sat							Sat	Sat	
	Sat/Sun							Sun	Sun	
6	**Sun/Mon**							Mon	Mon	
	Mon/Tue							Tue	Tue	
	Tue/Wed							Wed	Wed	
	Wed/Thu							Thu	Thu	
	Thu/Fri							Fri	Fri	
	Fri/Sat							Sat	Sat	
	Sat/Sun							Sun	Sun	

Appendix 5: All sleep logs from Germany

Pink dots: Early types Yellow dots: Late types

Appendix 6: Cities >100,000 residents (2003, www.citypopulation.de)

Germany

Aachen	256,605
Augsburg	259,217
B. Gladbach	106,053
Berlin	3,388,477
Bielefeld	328,452
Bochum	387,283
Bonn	311,052
Bottrop	120,324
Braunschweig	245,076
Bremen	544,853
Bremerhaven	118,276
Chemnitz	249,922
Cottbus	107,549
Darmstadt	139,698
Dortmund	589,661
Dresden	483,632
Duisburg	506,496
Düsseldorf	572,511
Erfurt	201,645
Erlangen	102,449
Essen	589,499
Frankfurt (M)	643,432
Freiburg (Br)	212,495
Fürth	111,892
Gelsenkirchen	272,445
Gera	106,365
Göttingen	122,883
Hagen	200,039
Halle (Saale)	240,119
Hamburg	1,734,083
Hamm	184,961
Hannover	516,160
Heidelberg	142,959
Heilbronn	120,705
Herne	172,870
Hildesheim	103,245
Ingolstadt	119,528
Jena	102,634
Karlsruhe	282,595
Kassel	194,322
Kiel	233,039
Koblenz	107,608
Köln	965,954
Krefeld	238,565
Leipzig	497,531
Leverkusen	161,543
Lübeck	212,754
Ludwigshafen	162,836
Magdeburg	227,535
Mainz	185,532
Mannheim	308,353
Moers	107,903
Mön.gladbach	262,391
Mülheim (R)	170,745
München	1,247,873
Münster	269,579
Neuss	152,050
Nürnberg	493,553
Oberhausen	220,033
Offenbach (M)	119,208
Oldenburg	158,340
Osnabrück	165,517
Paderborn	141,800
Pforzheim	119,046
Potsdam	144,979
Recklinghsn.	123,144
Regensburg	128,604
Remscheid	117,717
Reutlingen	112,346
Rostock	198,303
Saarbrücken	181,860
Salzgitter	109,855
Siegen	107,768
Solingen	164,543
Stuttgart	589,161
Trier	100,180
Ulm	119,807
Wiesbaden	271,995
Witten	101,823
Wolfsburg	122,724
Wuppertal	362,137
Würzburg	132,687

Austria:

Graz	226,244
Innsbruck	113,392
Linz	183,504
Salzburg	142,662
Wien	1,550,123

Switzerland:

Basel	165,051
Bern	122,707
Genève	177,535
Lausanne	116,332
Zürich	342,518

Appendix 7: SPSS Syntax

Syntax contains no data. Different sets of data were used for Factor analysis and Multiple Regression & ANCOVA, dependent on variables included.

Factor Analysis (Principle component analysis, PCA)

```
FACTOR
  /VARIABLES bt_w so_w se_w iwu_w fa_w dip_w ms_w sld_w bt_f so_f se_f iwu_f fa_f dip_f ms_f sld_f sld_a    msf_sc
 /MISSING LISTWISE /ANALYSIS bt_w so_w se_w iwu_w fa_w dip_w ms_w sld_w bt_f so_f se_f iwu_f fa_f dip_f ms_f sld_f
sld_a ms_fsc
  /PRINT UNIVARIATE INITIAL CORRELATION SIG DET KMO INV REPR AIC EXTRACTION ROTATION
  /CRITERIA MINEIGEN(1) ITERATE(25)
  /EXTRACTION PC
  /CRITERIA ITERATE(25)
  /ROTATION VARIMAX
  /METHOD=CORRELATION .
```

Multiple Regression

Note: separate analyses were performed for each dependent variable (bold)

```
REGRESSION
  /DESCRIPTIVES MEAN STDDEV CORR SIG N
  /MISSING LISTWISE
  /STATISTICS COEFF OUTS CI BCOV R ANOVA COLLIN TOL CHANGE ZPP
  /CRITERIA=PIN(.05) POUT(.10)
  /NOORIGIN
  /DEPENDENT ms_fsc / sld_a / ms_f / ms_w / sld_f / sld_w
  /METHOD=STEPWISE age gender  /METHOD=STEPWISE photo lat adole /METHOD=STEPWISE por bmi
  /SCATTERPLOT=(*ZRESID ,*ZPRED )
  /RESIDUALS DURBIN HIST(ZRESID) NORM(ZRESID)
  /CASEWISE PLOT(ZRESID) OUTLIERS(3) .
```

Analysis of Covariance (ANCOVA)

Note: separate analyses were performed for each dependent variable (bold)

```
UNIANOVA
  ms_fsc / sld_a  BY gender age por photo  WITH bmi adole
  /CONTRAST (gender)=Simple(1) /CONTRAST (age)=Simple(1) /CONTRAST (por)=Simple(1) /CONTRAST (photo)=Simple(1)
  /METHOD = SSTYPE(4)
  /INTERCEPT = INCLUDE
  /EMMEANS = TABLES(gender) WITH(bmi=MEAN adole=MEAN) COMPARE ADJ(SIDAK)
  /EMMEANS = TABLES(age) WITH(bmi=MEAN adole=MEAN) COMPARE ADJ(SIDAK)
  /EMMEANS = TABLES(por) WITH(bmi=MEAN adole=MEAN) COMPARE ADJ(SIDAK)
  /EMMEANS = TABLES(photo) WITH(bmi=MEAN adole=MEAN) COMPARE ADJ(SIDAK)
  /EMMEANS = TABLES(gender*age) WITH(bmi=MEAN adole=MEAN)
  /EMMEANS = TABLES(gender*por) WITH(bmi=MEAN adole=MEAN)
  /EMMEANS = TABLES(age*por) WITH(bmi=MEAN adole=MEAN)
  /EMMEANS = TABLES(gender*age*por) WITH(bmi=MEAN adole=MEAN)
  /EMMEANS = TABLES(gender*photo) WITH(bmi=MEAN adole=MEAN)
  /EMMEANS = TABLES(age*photo) WITH(bmi=MEAN adole=MEAN)
  /EMMEANS = TABLES(gender*age*photo) WITH(bmi=MEAN adole=MEAN)
  /EMMEANS = TABLES(por*photo) WITH(bmi=MEAN adole=MEAN)
  /EMMEANS = TABLES(gender*por*photo) WITH(bmi=MEAN adole=MEAN)
  /EMMEANS = TABLES(age*por*photo) WITH(bmi=MEAN adole=MEAN)
  /EMMEANS = TABLES(gender*age*por*photo) WITH(bmi=MEAN adole=MEAN)
  /PRINT = DESCRIPTIVE ETASQ PARAMETER HOMOGENEITY LOF GEF
  /CRITERIA = ALPHA(.05)
  /DESIGN = bmi adole gender age por photo gender*age gender*por age*por gender*age*por gender*photo age*photo gen-
der*age*photo por*photo gender*por*photo age*por*photo gender*age*por
```