Aus dem Institut für Medizinische Psychologie
Vorstand: Prof. Martha Merrow

Of ‘ Islands and Pancakes’: The Shape of Sleep in Shift Work

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Dorothee Fischer

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Berichterstatter:	Prof. Dr. Till Roenneberg
Mitberichterstatter:	Prof. Dr. Axel Steiger
	Prof. Dr. Christoph J. Lauer
	Priv. Doz. Dr. Jan Rémi
Mitbetreuung durch den promovierten Mitarbeiter:	Dr. Céline Vetter
Dekan:	Prof. Dr. med. Dr. h.c. M. Reiser, FACR, FRCR
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Er beschloss,
sein Leben zu ändern,
die Morgenstunden auszunutzen.
Er stand um sechs Uhr auf,
duschte, rasierte sich, kleidete sich an,
genoss das Frühstück,
rauchte ein paar Zigaretten,
setzte sich an den Arbeitstisch und
erwachte am Mittag.

- Ennio Flaiano: *Diario notturno.* (1956).

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0 Foreword

Dear reader,

I can see no reason for why a dissertation should not have a foreword encouraging the reader's interest and guiding her and his expectations. Speaking of, you might have hoped for a travel guide of picturesque Caribbean beaches, or family recipes of delicious desserts, or even how to get your body shaped while sleeping. I was told there are doctoral theses on those topics, and in case you picked the wrong one, I promise I won't be offended by your closing this document. Yet, and this I promise, too, if you go on reading, you will definitely learn something about shapes and sleep, about islands and pancakes, and you might even learn something about why you like pancakes most on Sundays.

If you think 'Chronotype' is the newest edition of Swiss watches or a monoCHRONatic light source, I must disappoint you but there is no need to be blue about it; yet, you may want to read the General Introduction… Otherwise, you can skip the introductory part with clear conscience, as each Project provides enough detail to understand its story including a brief summary. At the core of this dissertation is the intent to optimize work schedules such that sleep-wake behaviour is as little affected as possible. The recurrent (or oscillating) theme is the disruption of sleep and circadian rhythms in shift work (if you think 'circadian' is the name of a celebrity's child, you might also want to read the Introduction). Although most people in the field seem to know what circadian disruption is and agree that it has potentially adverse effects on health and safety, only few explicit definitions and even fewer measures exist.

Project One shows the importance of individual (*i.e.* chronotype) and operational characteristics (*i.e.* shift sequence) for circadian disruption, two crucial features often underestimated in shift work research. In **Project Two**, a new approach for quantifying circadian disruption of the sleep-wake cycle is proposed, involving both, individual chronotype and shift sequence. Last, **Project Three** challenges current guidelines on recommended number of consecutive (night) shifts applying the newly developed measure for circadian disruption.

It is not my intent to disrupt this foreword as I could endlessly continue writing on it. But in your own interest, I will stop here.

I hope reading this work will neither ruin your appetite for pancakes nor the desire for island travels; yet, I hope it will raise your understanding of individual and time–dependent differences in appetite and desire.

Sincerely,

Dorothee Fischer

1 General Introduction

1.1 What are clocks (for)?

As a privilege of modern times and Western industrialisation, many of us know the 'jet lag malaise' induced by fast travels across time zones. Sleepiness, digestion problems, and cognitive impairment are common symptoms of what happens when the body is not in synchrony with external time.[1] Twice a year in several countries, we experience a 'mini jet lag' caused by transition into and out of daylight saving time that pushes us back in seasonal progression by four and six weeks in spring and autumn, respectively.[2] Increasingly more of us engage in permanent or rotational shift work experiencing chronic or 'social jet lag' by sleeping, eating and being active when the body is oppositely tuned.[3] What all three phenomena have in common is the disruption of internal, biological rhythms, most obviously that of the daily sleep-wake cycle. Life is embedded into a temporal structure of light and dark, of warm and cold, created by the earth's 24-h rotation, and so is our biology. Anticipating cyclic environmental changes, both, within a day and over the year, permits organisms to occupy not only spatial and social niches in ecology, but also temporal ones. In millions of years of evolution, all living organisms have evolved a mechanism enabling them to measure time and predict periodic changes – the circadian clock, governing behaviour and physiology in bacteria, plants and animals, including humans.

The word *circadian* (lat. 'circa' – about, 'dies' – a day) had been coined by Franz Halberg to emphasise that the periodicity of the internal clock is 'approximately one day' rather than precisely 24h.[4] When shielded from environmental time cues and kept in constant conditions, the circadian clock 'free-runs' generating an endogenous day that is slightly longer than 24h, averaging 24h 11min ± 8 min in humans.[5] 'Free-running' refers to the

capability of the clock to display a self-sustained rhythm and to continue oscillating with an intrinsic period *tau* (τ) in the absence of external cues. It took centuries of research to proof that observed behavioural and physiological rhythms were endogenously generated rather than the result of a mere response to external periodic changes. Today, a free-running rhythm with a cycle deviant from 24h in constant conditions is considered one of three major characteristics of circadian clocks ('self-sustainability').[A]

The human circadian clock resides in the brain few centimetres behind the root of the nose, in a pair of nuclei above the crossing of the optic nerves ('optic chiasm').[6] The 'suprachiasmatic nuclei' (SCN, also used in singular form) are bilaterally located in the anterior part of the hypothalamus and comprise approximately 20.000 neurons, equalling the size of two grains of rice. The SCN exhibit a self-sustained rhythm in firing rate with a circadian period τ. If SCN tissue is cross-transplanted between two animals, the donor's specific period is transmitted to the recipient, *e.g.,* as reflected in its activity-rest cycle.[7] In order to adjust its endogenous circadian cycle length to precisely the 24h period of the external solar day, the SCN receives light input from the retina via a direct axonal pathway, the retinohypothalamic tract.[8] Unlike other vertebrates, photoreceptors in mammals are exclusively ocular,[9] although extra-retinal photoreceptors have been proposed to exist, for example in skin behind the human knee,[10] a finding that could never be replicated by other research groups.[11] Mice lacking all retinal photoreceptors known at that time (rods and cones) failed to show visual responses to light, but could still entrain to photic stimuli, indicating that a new non-rod, non-cone and non-image forming photoreceptor was responsible for entraining the circadian system.[12] Retinal ganglion cells appeared a proper candidate, and

[A] Another crucial feature of the circadian clock is 'temperature compensation', revealed by Colin Pittendrigh, a pioneer of circadian research, and his experiments on the fruit fly *Drosophila pseudoobscura* (1966). A functional clock must compensate for temperature differences that otherwise would accelerate or decelerate chemical processes and consequently alter their period.

studies revealed that ~1% of all retinal ganglion cells are light responsive expressing the photopigment melanopsin ('intrinsically photosensitive retinal ganglion cells', ipRGCs).[13,14] In mice deficient of rods and cones and melanopsin, all responses to light are lost.[15] Yet, melanopsin-knockout mice could still entrain to a light-dark regimen, suggesting that image-forming receptors may as well play a role in photic entrainment.[16] The spectral sensitivity of ipRGCs peaks in the short wavelength range,[17,18] and the action spectrum for light-induced melatonin suppression identified 446 – 477 nm as the most potent wavelength region revealing blue light as most influential for the circadian system.[19]

The fact that the SCN oscillate endogenously raises the question for the underlying mechanism. How do they generate a self-sustained circadian rhythm? In 1971, three mutants of the fruit fly Drosophila melanogaster were identified (one with a long period of 28h, one with a short period of 20h, and one arrhythmic phenotype) that could be traced back to the same gene, named *Per* for period.[20] With this the first clock gene was discovered, and others followed leading to the proposal of a potential molecular mechanism in 1990: the transcriptional-translational feedback loop.[21] The basic idea is that a gene is transcribed in the nucleus of a cell, and then translated into a protein at the ribosome in the cytoplasm followed by the entrance of the protein into the nucleus where it represses its own transcription. Finally, when the protein is degraded, the cycle restarts. Initially proposed as a simple negative feedback loop, we now know that in mammals the generation of an endogenous rhythm at the molecular level involves a complex network with interlocking circuits and several genes (*i.e. Per 1*, *2* and *3*, *Cry*(ptochrome) *1* and *2*, *Clk* (Clock), *Bmal1*, *Rev-erbα*, and *Dec 1* and *2*).[22,23] The SCN with its remarkable qualities appears the master pacemaker organising the daily temporal structure of bodily processes. Yet, it turned out that self-sustained oscillators exist in single cells throughout the body expressing similar sets of

clock genes though they need periodical inputs from the SCN to prevent the spontaneous dampening of their rhythmical activity with time.[24,25]

Together, the central clock and the peripheral oscillators form a hierarchical circadian system regulating and modulating physiological properties as manifold as gene expression, hormone secretion and mental performance. The notion of the circadian organisation as a temporal program, a time-keeping system thus appears more appropriate than the idea of 'one' circadian clock.

1.2 The Art of Entrainment[B]

In order for the organism to accurately predict and thus be prepared for periodic environmental changes, the circadian system needs to be synchronised with the external light-dark cycle as the alternation of light and darkness provides the most precise and reliable signal in the environment for time of day. Besides self-sustainability and temperature compensation, 'entrainability' is another major feature (if not 'the' feature) of circadian clocks. Entrainment derives from the French word 'entraîner' meaning 'carry over' or 'sweep along' in English. It describes the synchronisation of the circadian clock to daily environmental changes, *e.g.,* the light-dark alternation created by the earth's 24-h rotation. Yet, by simply responding to external changes, any time-responsive system could be passively synchronised. In contrast, entrainment is an active process during which the circadian system as a self-sustaining oscillator itself assumes a specific phase relationship with an external rhythm that is able to reset the clock.[26] Any periodic signal that can reset and shift the clock is called 'zeitgeber' (from the German word for 'time giver').[27] The phase angle between two rhythms is called *phase of entrainment* Ψ and relates the reference point of an internal circadian rhythm with that of the external zeitgeber cycle (*e.g.,* relative timing of core body

[B] Title from: Roenneberg, Daan & Merrow, 2003.[26]

temperature minimum and dawn).[26]

The slight deviations of free-running rhythms from 24h have sometimes led to the argument that entrainment of the clock is needed to correct for that deviation. Yet, constant conditions producing free-running rhythms are never encountered in real life, as the rotation of the earth and its non-perpendicular North-South axis cause daily and seasonal variability in photoperiod. Thus, the mechanisms behind a functional clock have evolved in a frequently alternating environment. Accordingly, the circadian clock is not entrained because its free-running rhythm is not exactly 24h but it free-runs with a *circadian* period to enable optimal functioning when entrained.[26,28] Furthermore, to serve its function, a circadian rhythm must run at the frequency of the earth's rotation as well as maintain a specific phase relationship to the solar cycle.[29] The phase of entrainment is not fixed but depends on several parameters: the endogenous period τ, the external zeitgeber period T, its light:dark ratio (photoperiod), as well as amplitude (strength) of the zeitgeber rhythm. Light turned out to be the predominant zeitgeber for circadian rhythms across the animal and plant kingdom, although other modalities were shown to have entraining qualities as well (*e.g.,* temperature in the Squirrel monkey,[30] barometric pressure in pocket mice,[31] social interaction in bats[32]). The dominance of sun time over social time as a zeitgeber in humans was shown in a large-scale online study in Germany, where people were asked about their sleep-wake behaviour on workdays and work-free days, and provided information on their geographical location.[33] Within the German time zone (GMT +1), averaged sleep timing on work-free days delayed from East to West by four minutes per longitude, which is exactly the time the earth needs to turn against the sun, or, respectively, the sun to rise at the next longitude.

1.2.1 Models of Entrainment

Here, three major approaches will be presented that have been proposed to predict how circadian clocks entrain to external light:dark cycles. All three models are so-called 'phase-only models' as their only read-out is phase, and all three models agree that stable entrainment is reached when the difference between internal and external period is somehow corrected for. Yet, they postulate different response characteristics to do so.

1.2.1.1 Non-parametric Entrainment

Colin Pittendrigh, one of the field's pioneers, approached entrainment by systematically investigating responses to transient light pulses while keeping animals in constant darkness.[34] From his experiments, he concluded that the system is instantaneously shifted to a new phase when perturbated by a light stimulus. Thus, stable entrainment is reached when the discrete phase shift ($\Delta\phi$) corrects for the deviation of the endogenous cycle length (τ) from 24h (T), that is $\Delta\phi = \tau - T$. The system's response will depend on when the stimulus is given, summarised in *phase response curves* (PRCs) graphing the magnitude of a phase shift as a function of circadian phase. PRCs for brief light pulses are characterised by a phase delaying and a phase advancing part usually separated by a 'dead zone' where no or insignificant phase shifts are seen. This approach was termed non-parametric entrainment as it assumes discrete instantaneous phase resetting in response to transient light pulses leaving the oscillator itself unaltered. A PRC for light was also demonstrated in humans revealing a characteristic type-1 PRC.[35] Type-0 and type-1 PRCs were first distinguished by Arthur Winfree who plotted the new phase to which an oscillation is reset as a function of the old phase (*phase transition curves*, PTCs).[36] If the stimulus is below a critical stimulus length (*i.e.* < 50 sec), one can reset to any phase by proper choice of when the stimulus is given resulting in a PTC with slope of 1 (hence, type-1 resetting) (Fig. 1.1a). In contrast, if the stimulus exceeds the critical duration, or the system is highly

sensitive to the perturbation, one can reset only to a limited range of new phases irrespective of the old phase (resulting in a PTC with slope of 0, thus type-0 resetting) (Fig. 1.1b). Winfree also predicted a *singularity* (*i.e.* the critical stimulus given at a specific phase) that stopped the oscillation, which he later indeed found experimentally.[36]

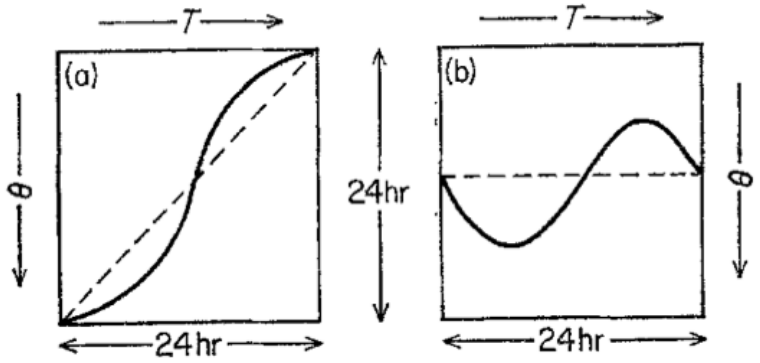

Figure 1.1. Idealised phase transition curves (PTCs) to low intensity (a) and high intensity stimuli (b). (a) Type-1 resetting, stimulus S < 50 sec. (b) Type-0 resetting, stimulus S > 50 sec. T represents the old phase at which the stimulus is given, θ indicates the new phase to which the rhythm is reset. *Source*: Winfree, 1970.[36]

1.2.1.2 Parametric Entrainment

A parametric approach to entrainment was proposed by Jürgen Aschoff, another pioneer of the circadian field, to account for effects of continuous light exposure rather than single light pulses.[37] He postulated that stable entrainment was achieved by changing the clock's velocity, and consequently changing phase, in order to match internal and external cycle length. *Velocity response curves* (VRCs) are estimated from PRCs and obtained similarly using responses to light compared with constant darkness. Essentially, parametric and non-parametric entrainment differs only by the nature of the light stimulus (*i.e.* transient or continuous).[38] A fundamental problem that PRCs (and with them VRCs) face is the assumption of a stable *tau* and P(V)RC itself. This view is challenged by the observation that *tau* changes dependent on zeitgeber conditions (*i.e.* after-effects of entrainment,[39,40] and

intensity of constant light[41]). Thus, model predictions for naturalistic entrainment should optimally be based on responses of synchronised rhythms.

1.2.1.3 An Integrated Approach to Entrainment

Roenneberg et al. have suggested a model of entrainment that is accessible from data under synchronised conditions.[38] Their concept does not assume mechanisms by which internal and external cycle lengths are matched (*i.e.* phase shifts, velocity changes) but integrates effects of light at different internal times as formalised by a *circadian integrated response characteristic* (CIRC). The CIRC assumes that light around subjective dawn compresses the internal cycle (resulting in phase advance), light around subjective dusk expands it (leading to phase delay), and a 'dead zone' separates both these parts (Fig. 1.2). Its form is determined by two factors: the asymmetry of the compressing and expanding regions, and the extent of the dead zone (making the CIRC more or less sinusoidal). Light exposures of any form (from skeleton photoperiods to extended and multiple light signals) are integrated over one cycle, and stable entrainment is reached when $\tau = T$, *i.e.* the internal period adjusts to the external cycle length via compression or expansion.

Figure 1.2. Circadian Integrated Response Characteristic (CIRC). The response characteristic includes a compressing part that is followed by a dead zone and a subsequent expanding part. It predicts how much a given light exposure will affect internal cycle length at different internal times. *Source*: Roenneberg et al., 2010.[38]

The CIRC makes important predictions regarding the phase of entrainment. In case $\tau = T$, phase of entrainment is independent of zeitgeber amplitude, because the proportions of compressing and expanding regions are symmetrical and therefore equally affected (Fig. 1.3 A and D). If $\tau > T$, the phase of entrainment moves to a later external phase thereby producing an integrated response that compresses the internal cycle length. Accordingly, modelling $\tau < T$ results in an earlier phase of entrainment, as now more of the expanding parts need to be exposed to light. In both cases, zeitgeber strength will affect phase of entrainment. With increasing amplitude of the zeitgeber cycle, the distribution of different phases of entrainment (resulting from $\tau >$ or $< T$) within a population will become narrower. Early phases will be delayed by a stronger zeitgeber, as the expansion portion increases more than its counterpart. Similarly, late phases will be advanced due to a larger increase of the compressing compared with the expanding part (Fig. 1.3 B and E, C and F). Notably, recent studies in humans and birds have confirmed those predictions.[42,43]

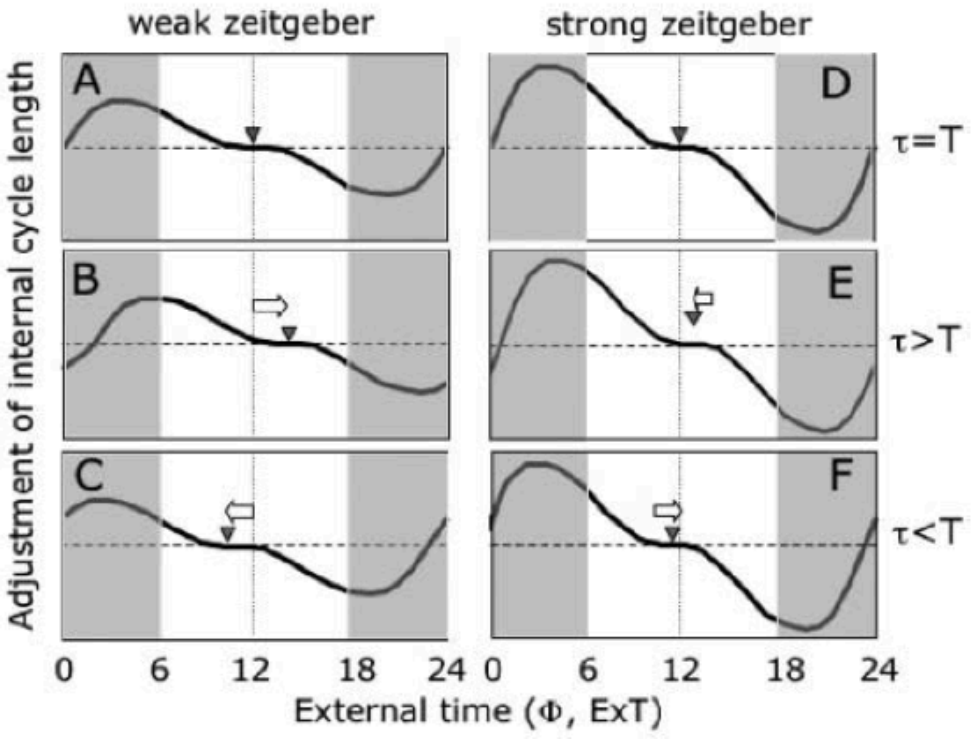

Figure 1.3. Entrainment scenarios with different conditions (weak and strong zeitgeber) and varying internal cycle lengths (τ). Phase of entrainment is indicated by the triangles (representing internal midday, InT 12). The white arrows show the direction in which the CIRC has "moved" in order to achieve stable entrainment (left panels: compared with (A); right panels: compared with the weak zeitgeber condition). *Source*: Roenneberg et al., 2010.[38]

Moreover, the CIRC predicts that two individuals with identical τ will assume different phases of entrainment under strong and weak zeitgeber conditions. Accordingly, two individuals with different τ can assume the same phase of entrainment under strong and weak zeitgeber conditions (Fig. 1.4). Therefore, it is of crucial importance that individual light exposure is concomitantly assessed if one aims at distinguishing between individuals who are entrained with a (more or less) late phase irrespective of zeitgeber conditions and those who are entrained late due to decreased zeitgeber strength. Circadian phenotypes thus differ not only from individual to individual, but also from environment to environment.[33]

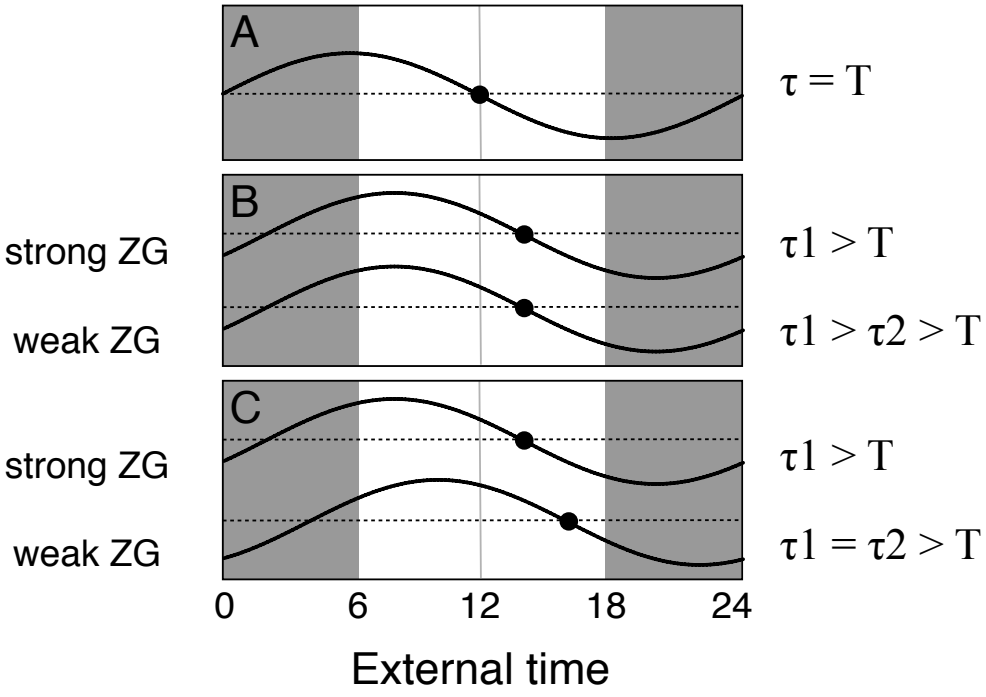

Figure 1.4. Exemplified CIRCs illustrating how zeitgeber conditions (weak *vs.* strong) and internal cycle length (τ) interact on phase of entrainment (black dots, representing internal midday, InT 12). (A) External and internal midday coincide when both cycle lengths match. (B) If zeitgeber conditions differ appropriately, two individuals can assume the same phase despite different τ. Accordingly, they can entrain differently albeit identical τ, by proper choice of zeitgeber conditions.

The phase of entrainment determines when physiological, behavioural and cognitive processes reach their peaks and troughs. Accordingly, individuals who entrain differently also vary in the timing of their biological functions. These differences are commonly called 'chronotypes'. In view of industrialisation, urbanisation, and electrical lighting, the impact of zeitgeber strength on the phase of entrainment is of great importance, and the resulting variation between individuals (*i.e.* 'chronotypes') appears equally challenging and promising for a 24/7 society.

1.3 Chronotypes – not a category

Various names have been appointed to chronotype, often used synonymously: morning and evening sleepers,[44] morning (M-types) and evening types (E-types),[44] early birds or morning larks and night owls,[45,46] morning and evening chronotypes,[47] diurnal[48,49] and circadian preferences,[50,51] and diurnal and circadian types.[52] They all refer to chronotype as a 'circadian phenotype',[53] *i.e.* the behavioural manifestation of underlying circadian rhythms, but they differ with respect to the behaviour (*e.g.,* sleep, physical activity, work hours, eating habits), which is partly due to how studies assess chronotype. A large body of studies demonstrates that chronotype reflects the relative timing of several physiological and behavioural processes, such as core body temperature, melatonin, cortisol, subjective alertness, electrodermal activity, cognitive performance, and most recently, clock gene expression (*Per1*, *Per2*, and *Rev-erba*).[44–46,54–56] According to circadian principles, the relative timing of events within the 24-h day depends on how the clock embeds itself into (entrains to) the external zeitgeber cycle. Thus, chronotype have been proposed to constitute the phase of entrainment Ψ of an individual.[38,57,58] For the purpose of this dissertation, the definition of chronotype follows this conceptualisation. As was outlined in a previous section (see 1.1), the notion of a temporal multi-oscillatory system appears more accurately than the idea of 'one' circadian clock. Consequently, there is not 'one' phase of entrainment but each oscillator assumes a specific phase relationship with the zeitgeber rhythm. The internal phase relationships between different circadian outputs are not necessarily fixed, and thus, chronotype will vary according to the assessed (internal and external) rhythms. Furthermore, the rhythm of interest can be characterised by different parameters (*e.g.*, maximum, minimum). It is therefore crucial to measure Ψ (chronotype) with respect to the outcome one is interested in.

1.3.1 Assessment of Chronotype

Several approaches exist to determine phase of entrainment at different levels (single cell, organ tissue, organism; central *vs.* peripheral), with different methods (invasive, non-invasive), and in various settings (in vitro, in vivo; field vs. laboratory) (for an overview, see[59]). In real life settings, self-reported questionnaires and sleep logs, as well as recordings of locomotor activity ('actimetry') are most commonly used, but clock-regulated hormones (*e.g.,* melatonin, cortisol) and clock gene expression can also be assessed in semi-controlled conditions where participants collect their own samples (*e.g.,* urine, saliva, buccal mucosa cells) at night under dim-light. Multiple chronotype questionnaires are available including the Morningness-Eveningness Questionnaire (MEQ),[60] the Composite Scale of Morningness (CSM),[61] the Circadian Type Questionnaire,[52] and the Diurnal Type Scale (DTS),[62] to name only some. The first und most widespread questionnaire is the MEQ containing 19 items with regards to habitual arising and bed times, preferred times of physical and mental performance, and subjective alertness after arising and before going to bed. The scores range from 16 to 86, with original cut-off values of 16 – 41: evening type, 42 – 58: neither type, and 59 – 86: morning type, that have later been revised to match the distributions observed in middle-aged people.[63] Yet, several aspects are critical. The classification of 'intermediate' or 'neither types' can result from two different response patterns: once by consistently ticking the intermediate box (values of 2 and 3), and once by choosing extreme but opposing categories (values of 0 and 4) eventually adding up to an intermediate overall score. Furthermore, chronotype as research variable aims at detecting differences in the temporal organisation of individuals and related variables. As such, it represents an internal time scale that arbitrary MEQ scores cannot be plotted on. Most importantly, the MEQ does not distinguish workdays from work-free days (*e.g.,* "How alert do you feel during the first half hour after you wake up in the

morning?“). Yet, the answers might be very different, if questions were posed separately.

To overcome these difficulties, Roenneberg et al. have developed the Munich ChronoType Questionnaire (MCTQ[64]) that poses simple questions about one’s actual sleep-wake behaviour on workdays and work-free days, *e.g.,* bedtime, time when one is prepared for sleep, estimated time to fall asleep, wake-up time, get-up time, and use of alarm clock. Several parameters can be computed, such as sleep latency, sleep inertia, and sleep start and end. Chronotype is determined via the phase of entrainment Ψ of the sleep-wake rhythm with the light-dark cycle using mid-sleep on work-free days corrected for potential over-sleep (MSF_{sc}) as a proxy (Fig. 1.5). Mid-sleep is calculated by sleep onset plus half the sleep duration (*i.e.* sleep from 1:00 a.m. to 9:00 a.m. results in a mid-sleep of 5:00 a.m.). It was shown to be a better phase marker than sleep start or wake-up time by means of predicting dim-light melatonin onset (DLMO), which is considered a direct phase marker of the circadian clock.[56,65]

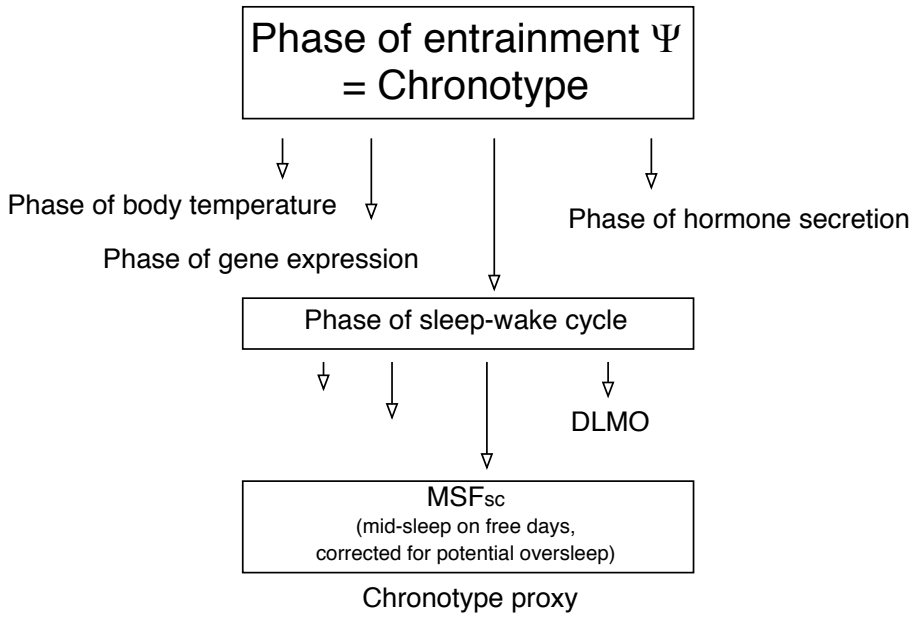

Figure 1.5. Chronotype as phase of entrainment Ψ can be determined via several output rhythms (*i.e.* sleep-wake rhythm), which in turn can be assessed using different phase markers as chronotype proxies (*i.e.* MSF_{sc}).

The correction for potential oversleep is applied to account for the finding that most people accumulate a certain sleep debt during their workweek due to early start times that they eventually compensate for by sleeping in on weekends (for correction algorithm, see supplemental data to Ref.[33]).[57] The resulting chronotype distribution for the German population is bell-shaped and slightly skewed to the left, revealing that most people are in between the rare extremes of early and late types (Fig. 1.6).[57] Most frequently, corrected mid-sleep falls between 4:00 and 4:30 a.m. with ~35% sleeping earlier and ~50% sleeping later. Assessing chronotype via mid-point of sleep results in a local time, representing an internal time scale. The MSF_{sc} corresponds to internal midnight, thus allowing for transformations between circadian and zeitgeber time. It also permits the calculation of chronotype from self-reported sleep logs and actimetry data.

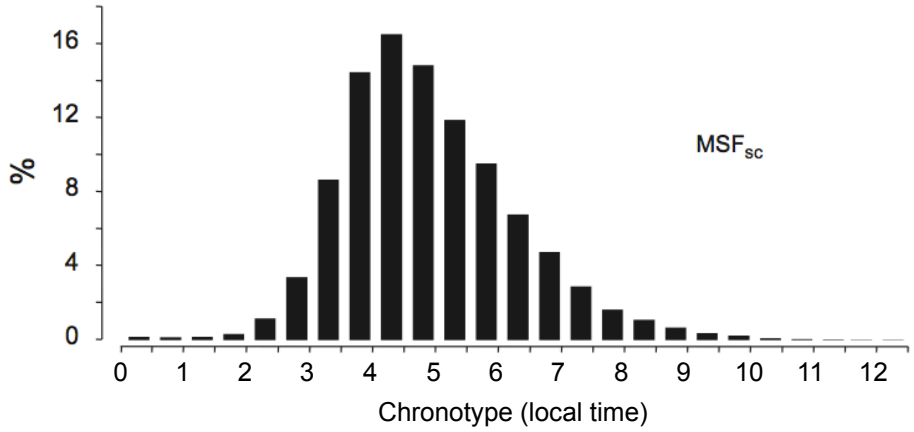

Figure 1.6. Chronotype distribution assessed with the Munich Chronotype Questionnaire (judged by MSF_{sc}, data collected mainly in Germany, Switzerland, the Netherlands, and Austria). *Source*: Roenneberg et al., 2007.[57]

1.3.2 Actimetry

Actimetry is a non-invasive method to monitor human rest-activity cycles. The device is usually worn continuously for several days around the wrist, ankle or waist, and records changes in acceleration as well as orientation (dual-axis

accelerometer). Thus, at rest relative to the earth's surface, the acceleration measured is 1 g (9.81 m/s^2) upwards. The mechanical motion is converted into an electric signal with a dimensionless final output, simply indicating more or less activity. Traditionally, time series in circadian research are 'double-plotted' to better visualise changes in period and phase over time. A double plot displays 'time' on both axes, yet in different solutions, with days on the ordinate and hours on the abscissa. The course of two subsequent days is shown in one row, and the last day is repeated in the next row, *i.e.* first row shows day 1 and 2, second row shows day 2 and 3, and so on. Figure 1.7 illustrates the double plot of locomotor activity ('actigraph') from a shift worker in a rotational 3-shift system (working morning, evening, and night shift).

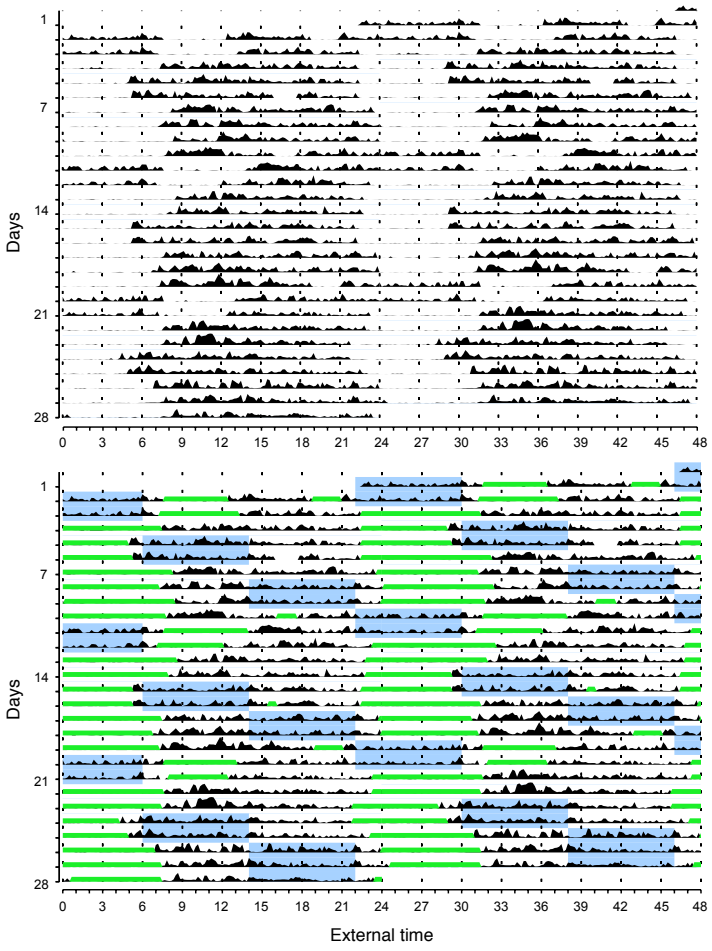

Figure 1.7. Actigraphs of a shift worker in a rotational schedule with morning, evening, night shifts showing raw activity data (upper panel), and extracted sleep bouts (green bars) and rotating working times (blue boxes) (lower panel). The data is double-plotted, *i.e.* first row contains day 1 (0 – 24h) and day 2 (24 – 48h), second row day 2 and day 3, third row day 3 and day 4, and so forth.

Several parameters can be computed from actimetry data. Usually, a cosine wave is fitted to the raw data applying a least square approach. Amplitude (A), range of oscillation ($RoO = 2A$), mean level or mesor (a rhythm-adjusted mean), period (P), frequency ($1/P$), and phase (φ or θ) can be derived and used for analyses. Additionally, the centre of gravity[66] can be calculated, a one- or poly-harmonic fit to the time series that, in case of a one-harmonic fit, corresponds to the acrophase (peak) of the rhythm. The centre of

gravity thus represents a phase marker of the rest-activity cycle, and can be visualised as follows: if the activity times series over 24h was plotted circularly on a piece of paper and cut out, then the centre of gravity would be the point at which a pin was put through the paper to fix it to the wall without further movements (Fig. 1.8). Finally, sleep parameters (onset, offset, duration, mid-point) can be extracted from actimetry by applying a two-step process involving a threshold-analysis and bootstrap correlation method. More detailed information on this process will be provided in Project Two (see 3.3.3), and a comprehensive description of methods assessing and analysing human activity and rest *in situ* by Roenneberg, Keller, Fischer, et al. is now in press.[67]

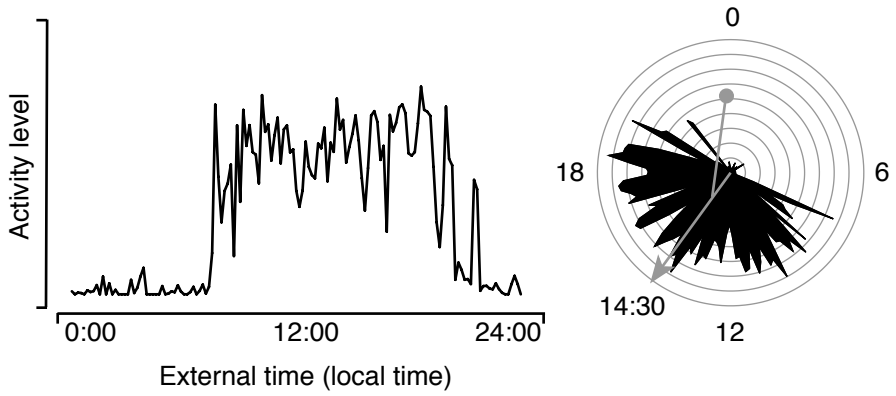

Figure 1.8. Centre of gravity. Raw activity time series is shown over the course of one day (line graph, left panel). When plotted on a circle (circumference indicating hours, radius showing level of activity), centre of gravity indicates the time point at which the black shape can be fixed to the wall by a pin representing a phase marker of the rest-activity cycle (right panel).

1.3.3 Sleep-wake Behaviour

Given its assessment via mid-sleep on free days (MSF_{sc}), chronotype strictly only represents Ψ of the sleep-wake cycle.[58] The alternation of sleep and wakefulness is one of the most overt rhythms in humans, and one of the most fascinating since its true function remains to be elucidated, though it likely serves the preparation of our brains to function optimally during wakefulness.[29] Several mathematical models of sleep-wake regulation have

been proposed, that can be classified into phenomenological models and physiology-based models.

1.3.3.1 Phenomenological Models

Classic phenomenological models focus on the interaction between sleep and circadian systems, without making assumption about linked physiological correlates. The first and highly influential model was proposed by Borbély[68] and later refined by Daan, Beersma and Borbély.[69] In their 'two process model' of sleep, the process 'S' reflects a homeostatic component that increases with wakefulness and decreases during sleep in an exponential manner, while process 'C' represents a wake-promoting circadian drive in the shape of a sinusoidal curve (Fig. 1.9). The process C opposes the homeostatic sleep propensity by peaking few hours prior to habitual bedtime in entrained humans and promoting wakefulness, and reaches its trough in the second half of the sleep episode when most of the sleep pressure is 'slept off' to ensure longer sleep. Thereby, the circadian modulation gates the homeostatically controlled sleep process consolidating it at a later circadian phase. Similarly, some hours before sleep is initiated, the nocturnal and sleep-promoting hormone melatonin is secreted in higher levels in the pineal gland, and subsequently suppresses via melatonin receptors in the SCN the wake-promoting signal, thereby enabling sleep directly after the circadian peak for wakefulness.[70] Kronauer et al. proposed another influential model of sleep-wake regulation.[71] Based on a van der Pol formalism, two interacting oscillators are coupled, one representing the self-sustained circadian pacemaker and the other the rest-activity cycle, successfully capturing the timing and duration of human sleep. The Kronauer model is still used in current models to represent SCN inputs, but accumulating insights into the neuroanatomy of sleep-wake control gave rise to mathematical models with a stronger physiological basis.

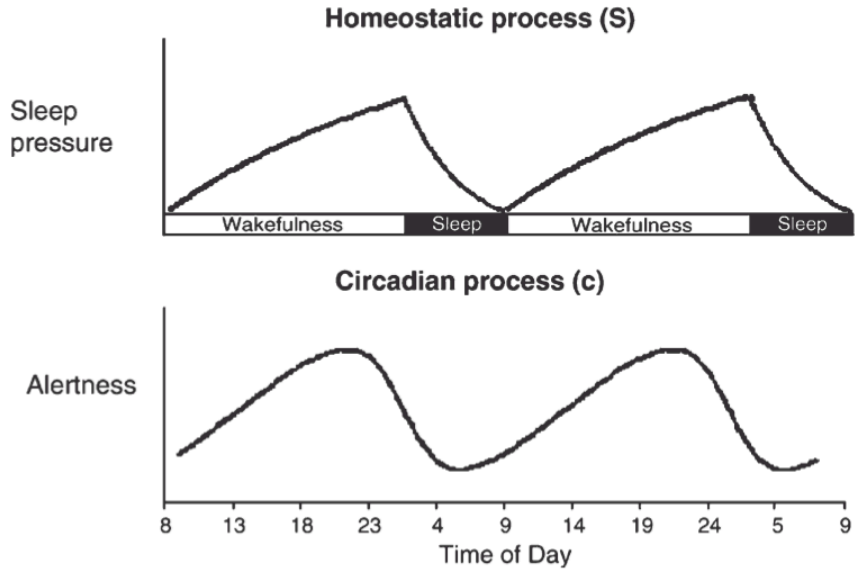

Figure 1.9. Two-process model of sleep regulation. The homeostatic process 'S' (sleep propensity) builds up during wakefulness and dissipates during sleep. The circadian process 'C' opposes the homeostatic sleep pressure by increasing the drive for wakefulness in a time-dependent manner. *Source:* Chellappa & Cajochen, 2009.[72]

1.3.3.2 Physiological Models

Physiology-based models specify interactions among brainstem and hypothalamic regions known to promote states of wakefulness, REM (rapid eye movement) sleep and non-REM (NREM) sleep, including their synaptic projections and respective neurotransmitters (for review, see[73]). They mathematically formalise current hypotheses regarding sleep-wake control, *e.g.,* the 'flip-flop switch' that drives rapid transitions between wakefulness and sleep by mutual inhibition,[74] and the 'reciprocal interaction hypothesis' describing how neuronal REM-on and REM-off populations generate ultradian NREM-REM cycles within sleep.[75] The following two paragraphs briefly summarize brain regions, neurotransmitters and synaptic projections commonly included in physiological models of the sleep-wake regulatory network.

1.3.3.2.1 Sleep-wake regulating centres in the brain

The wake-promoting populations include locus coeruleus with the neurotransmitter norepinephrine, serotonergic dorsal raphé nuclei, and histaminergic tuberomammillary neurons. Subpopulations of laterodorsal tegmental nucleus and pedunculopontine tegmental nucleus are considered both, wake and REM-promoting regions and involve the neurotransmitter acetylcholine. The NREM-promoting population comprises the ventrolateral preoptic area expressing the neurotransmitter GABA. Additionally, the suprachiasmatic nuclei are added to provide circadian modulation to the above-mentioned sleep- and wake-promoting populations. Thereby, circadian drive enters the models via a 24-h variation in SCN firing rate, and homeostatic sleep propensity is incorporated through the sleep-promoting population.[72,73,76]

1.3.3.2.2 Synaptic projections among neuronal populations

Mutually inhibitory projections between wake- and NREM-promoting regions reflect the “flip-flop switch” hypothesis driving rapid transitions between distinct states of sleep and wakefulness.[74] Within sleep, transitions between NREM and REM sleep stages are assumed to be regulated via interactions between REM-on (excitatory cholinergic populations involving tegmental nuclei) and REM-off regions (*e.g.,* the inhibitory monoaminergic wake-promoting locus coeruleus), as proposed by the reciprocal interaction hypothesis.[75] In models, these interactions are represented via excitatory projections from REM- to wake-promoting populations and inhibitory projections directed from wake- to REM-promoting areas. The SCN projects to locus coeruleus (wake) and tegmental nuclei (REM) (excitatory), and to the ventrolateral preoptic area (NREM) (inhibitory), standing for a simplified net effect of several indirect pathways and multiple transmitters that mediate SCN signalling to the sleep-wake regulating centres.[73]

Physiology-based models have been successfully applied to describe human sleep-wake behaviour under entrained and free-running conditions as well as in shift work settings.[77–79] Recently, using such a physiological model, the mechanisms of chronotype defined as an individual's preferred sleep-wake schedule have been explored raising the question for homeostatic influences on chronotype.[80,81]

1.3.3.3 Chronotype or Homeotype?[C]

The best-documented chronotype difference is that in sleep-wake behaviour with evening types sleeping later than morning types. Given the here followed definition of chronotype as the phase of entrainment Ψ of the sleep-wake rhythm with the light:dark cycle and its assessment by mid-sleep on days off (MSF_{sc}),[58,64] homeostatic components of sleep-wake regulation might also influence Ψ and thus, chronotype. Studies from Valérie Mongrain's lab at the Université de Montréal in Canada suggest that chronotypes may be divided into two subgroups: a 'non-congruent' subgroup comprising individuals with extreme, opposed chronotypes (assessed with MEQ) but overlapping, intermediate circadian phases (measured by dim-light melatonin onset, DLMO), and a 'congruent' subgroup with individuals showing both, extreme chronotypes and extreme DLMOs.[82–84] In subjects with intermediate DLMO times, morning types showed a higher initial level and a steeper decay rate of slow-wave activity in the frontal derivation during NREM sleep compared with evening types.[82] Given a similar circadian phase by means of melatonin onset, homeostatic parameters such as different dissipation levels of sleep pressure might account for the observed variation in sleep timing (*i.e.* chronotype).

[C] Term from: Chellappa & Cajochen, 2009.[69]

A quantitative modelling study showed that chronotype (again, defined as preferred sleep-wake schedule) depended on the relative influences of homeostatic and circadian drives.[81] Reducing circadian amplitude as well as period advanced sleep timing. Likewise, increasing homeostatic sleep propensity as well as the parameter representing adenosine concentration (a neuromodulator involved in sleep homeostasis[85]) caused earlier sleep times, whereas enhancing the homeostatic time constant for clearance and accumulation delayed the timing of sleep. The model thus offers potential mechanisms underlying the two above-describe chronotype subgroups. According to the model, in congruent individuals (DLMO and sleep timing are concordant), circadian period is assumed to cause earlier (shorter τ) or later sleep times (longer τ). In contrast, in non-congruent individuals (intermediate DLMO but advanced or delayed sleep times), differences in homeostatic kinetics are argued to account for morningness (shorter time constant) or eveningness (longer time constant).

When assessed via sleep-wake behaviour, chronotype seems to be naturally influenced by the interaction of both, circadian and homeostatic parameters, an assumption further underpinned by age-related changes in chronotype, circadian system and sleep regulation.[86]

1.3.4 Chronotype, Age and Sex

Chronotype is not a fixed trait, but influenced by multiple factors, such as genetics,[48,87] light exposure (or zeitgeber strength),[38,57] age, and sex (and/or gender).[53] From childhood throughout puberty and adolescence, young people grow later and later with women peaking in ‘lateness’ at the age of 19.5 years and men at around 21 years.[53] After that, chronotype advances with increasing age (Fig. 1.10). The differences between males and females of 1h on average persists until the age of ~50 years. Within each age group (binned in years between ages 12 – 60), the distribution resembles the bell-shape of the general population. The turning point of MSF_{sc} from steadily

increasing to steadily decreasing has been suggested a biological marker for the end of adolescence.[53] Yet, data were collected in a cross-sectional survey study, and cannot distinguish between an age-related effect and a cohort-specific effect (*e.g.,* people having become later over the past decades).[53,57] A recent study by Crowley et al. though showed in a 2.5-year longitudinal design that chronotype continuously delayed during this period, providing first (medium-term) support for a systematic age effect.[88]

Research on human circadian rhythms and aging consistently demonstrated that the phase of several physiological rhythms advances with increasing age, as was shown for body temperature, melatonin, cortisol, and blood pressure with a typical phase difference of about 1 h between young and old subjects.[89–92] Accordingly, studies examined age-related differences in period and amplitude of circadian rhythms, expecting older people to have shorter τ and shallower amplitude. However, results were mixed finding evidence both, in favour and against those assumptions (for a review, see[93]), leading to the hypothesis that the SCN may still generate high-amplitude rhythms but the downstream ability of the SCN to drive peripheral oscillators may attenuate with aging.[94]

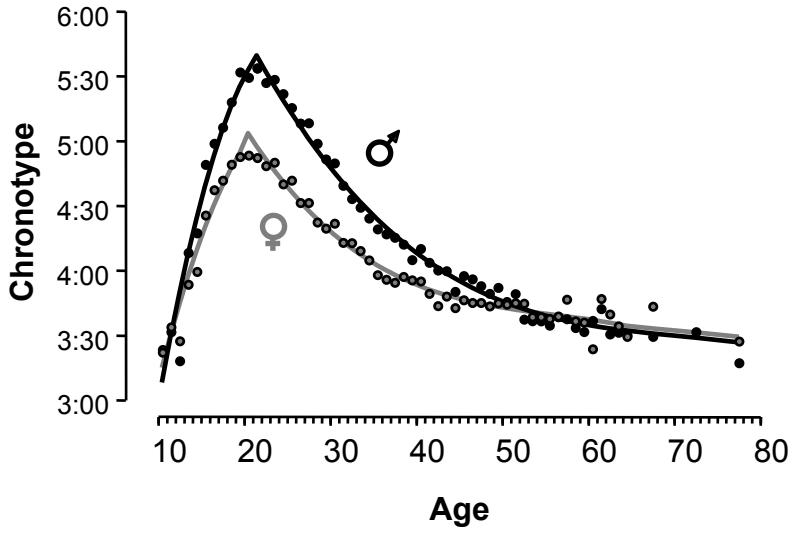

Figure 1.10. Chronotype (MSF_{sc}) is age- and sex-dependent. Grey line and dots = females. Black line and dots = males. *Source*: Roenneberg et al., 2004.[53]

1.3.5 Chronotype & Health

An ever-growing number of studies is finding effects of chronotype (usually assessed with questionnaires such as the MEQ) on physiological and psychological health with eveningness associating to increased risks. Examined outcomes range from self-reported morbidity,[95] via obesity,[96,97] type-2 diabetes and cardiovascular disease,[98] asthma[99] to substance abuse[3] and psychiatric diseases, such as depression,[100–102] bipolar[103,104] and eating disorders.[51] Several studies focused on the relationship between chronotype and behavioural and emotional problems in adolescents.[49,105–108] Most (but not all[49,106]) of these studies control for overall sleep duration to independently estimate the impact of chronotype, suggesting that an evening type *per se* promotes, *e.g.,* alcohol drinking, smoking, and lower school performance. In general, sleep duration appears to be independent from chronotype.[57,60] However, a striking relationship emerges if analysed separately for workdays and work-free days:[57,109,110] the later the chronotype, the shorter sleep duration on workdays, and the longer sleep duration on days off suggesting a socially-induced sleep debt most profound in late types (Fig. 1.11).[D] This

difference is not reflected in overall sleep duration, as the averaged weekly amount of sleep is similar across chronotypes. Wittmann and colleagues suggested the concept of 'social jetlag' to quantify this misalignment calculated as the absolute difference of mid-sleep on free days and work days (MSF – MSW).[3] They could show that alcohol drinking and smoking is likely due to social jetlag highest in late chronotypes. Furthermore, using mediation analysis, they demonstrated that only those late types who smoked and drinked suffered from a decreased psychological well-being, indicating that late types are at higher risk for emotional problems because of a socially-induced misalignment rather than being late *per se*.[111] In line with their findings, Roeser et al. developed a 'Chronotype Academic Performance Model' showing that academic performance was not related to chronotype but mediated by daytime sleepiness and learning motivation.[108] Moreover, a meta-analytic investigation on chronotype, cognitive abilities and academic achievement revealed that evening-types had higher cognitive abilities but performed worse whereas the reversed pattern was found for morning-types.[112]

Taken together, adverse health- and performance-related outcomes do not seem to attribute to a late chronotype as such but to the higher likelihood of late types to experience circadian misalignment and sleep loss on school and workdays. This is of key importance for interventions suggesting that external factors (*i.e.* school and work start times) rather than internal factors (*i.e.* chronotype, sleep timing) should be targeted.

[D] For those who have read the foreword (or might want to do this now): this is why pancakes are best on Sundays, because on free days alarm clocks are banished, internal and external time are in line, and so are appetite and food supply.

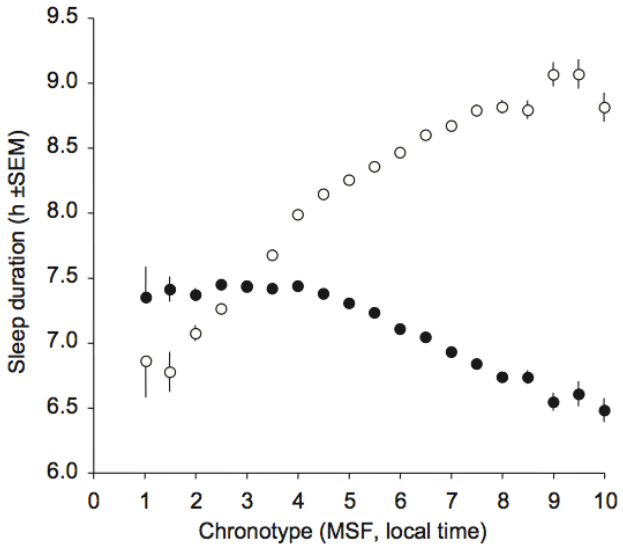

Figure 1.11. Chronotype and averaged sleep duration on workdays (filled circles) and work-free days (open circles). The gap widens the later the chronotype, indicating an increasing sleep debt. *Source*: Roenneberg et al., 2007.[57]

1.4 Circadian Disruption

Similar to chronotype, circadian disruption has many names, *e.g.,* circadian misalignment,[113] circadian desynchrony,[114] and chronodisruption.[115] The term refers to a mismatch either between external and internal time (*i.e.* 'external desynchrony') or among internal rhythms leading to altered internal phase relationships (*i.e.* 'internal desynchrony'). As a consequence, physiological processes take place at improper times potentially impairing general body functions and eventually entailing adverse impacts for health, such as metabolic and cardiovascular diseases.[116,117] Albeit circadian disruption is a widely used and accepted term thought to be causally involved in etiology and progression of several diseases, there is a lack of clear theoretical frameworks and quantifiable measures impeding the systematic investigation of its causes and consequences.[118] The disruption of biological rhythms becomes most obvious in shift work, where the normal phase-relationship between sleep-wake behaviour and other circadian rhythms is disturbed. Shift

workers need to eat, sleep and be active at times their circadian clock tells otherwise. As described in the previous section, the timing of biological processes varies according to the phase relationship that an individual assumes with the external solar day. Thus, the occurrence of circadian disruption will depend on the phase of entrainment (*i.e.* chronotype) determining when and to what extent endogenous functions are not in line with exogenous demands, such as in shift work.

1.4.1 Circadian Disruption in Shift Work

Approximately 20% of the work force in industrialised countries engages in shift work with an increasing trend.[119] Detrimental health effects are well documented showing an elevated risk in shift workers as compared with day workers for sleep disorders, gastrointestinal and metabolic pathologies (*i.e.* diabetes, obesity, metabolic syndrome, elevated cholesterol and triglycerides levels),[120–122] cardiovascular disease (*i.e.* hypertension, decreased heart rate variability, ischemic heart disease),[123–125] and also cancer (breast, colorectal, endometrial and prostate tumours).[126–129] In 2007, the International Agency for Research on Cancer (IARC) classified shift work that involves circadian disruption as probably carcinogenic to humans (Group 2A).[130] The classification was based on “limited evidence in humans for the carcinogenicity of shift-work that involves night work” and “sufficient evidence in experimental animals for the carcinogenicity of light during the daily dark period”. In six out of eight studies, epidemiological data showed modestly increased risks for breast cancer in female flight attendants and nurses working night shifts as compared with employees not engaged in night work. Animal experiments were carried out in nocturnal rodents examining effects of constant light, dim light at night, simulated chronic jet lag, or circadian timing of carcinogens demonstrating major increases in incidence or growth of tumours. Furthermore, ablation of pineal gland or reduced melatonin levels at night led to the same results. Eventually, these findings were congregated in

the 'light-at-night' hypothesis assuming a chain of arguments.[131,132] Oxygen radicals can damage DNA, which in turn can cause cancer. Melatonin as an indolamine potentially scavenges oxygen radicals, but its synthesis during the dark period can be suppressed by light. Thus, reduced nocturnal melatonin levels due to light at night are argued to cause cancer via increased DNA damage by oxygen radicals.[133,134] In this hypothesis, circadian disruption is assumed to occur through the suppressive effect of light on nocturnal melatonin. Besides other critical matters that are comprehensively summarized by Kantermann & Roenneberg,[135] it does not take into account the crucial aspect of inter-individual differences in biological time but defines the biological night as the "daily dark period".[130] However, if a shift worker is fully adjusted to the night shift, melatonin will be high in the morning and daylight will potentially suppress it. Accordingly, the degree of melatonin suppression by light will vary with circadian phase, and hence, chronotype. Also, bright light exposure during the day influences the magnitude of melatonin suppression at night (reduction)[136,137] as well as the secretion during the following night (increase).[137,138] Thus, it might not be acute suppression of melatonin by dim light at night, but dim light during the day that leads to a reduction of nocturnal melatonin levels.[135] Taken together, circadian disruption via light at (external) night will depend (amongst other factors) on (i) individual chronotype (defining internal night and timing of melatonin secretion), and (ii) individual light history, which in turn is co-determined by shift schedule.

1.4.2 Sleep-Wake Behaviour in Shift Work

Shift schedules can differ in respect to several features: duration of shift (8h *vs.* 12h), speed (number of consecutive shifts), direction (clockwise/ forward *vs.* counter-clockwise/ backward rotations), rest periods between shifts, distribution and number of work-free days, permanent *vs.* rotational, regular *vs.* irregular, and continuous vs. discontinuous (free weekends) schedules, to

name some.[139] In view of this immense variety, ergonomic guidelines have been established to advise designs for shift schedules that aim at minimising adverse effects on health and safety of shift workers.[140–142] These guidelines currently recommend, *e.g.,* forward-directed rotations, avoidance of early start for morning shifts (not before 6 a.m.), reduction of shift duration (≤ 12h), and limiting number of consecutive night shifts (≤ 3). The recommendations are primarily based on research on sleep and sleepiness, either subjectively assessed via questionnaires, or objectively using actimetry and polysomnography. In general, different types of shifts (morning, evening, night shift) are differentially associated with sleep duration, quality, and architecture. While evening shifts (standard European transition times 2:00 p.m. – 10 p.m.) appear least interfering, morning and night shifts are characterized by a drastically shortened duration of 2 – 4 h less sleep and reductions in stage 2 and REM sleep.[143–146] Interestingly, while slow wave activity seems to be unaffected during sleep after the night shift, a decrease was found for sleep before the morning shift that correlated with the anticipation of an early awakening.[147] Both, morning and night shifts are associated with napping due to higher levels of sleepiness.[148–150] About one-third of employees take a nap in the afternoon before the first night shift as well as a late afternoon nap between successive night shifts. Likewise, ~33% nap in the early afternoon following the morning shift, soon after returning home. After the last night shift, it usually takes two unrestricted sleep episodes before normal levels of sleepiness are achieved.[151] Also, electroencephalogram studies show involuntary short sleep episodes during the night shift summing up to ~40 min sleep.[152] However, particular shift schedule features appear to influence these ‘general’ sleep patterns. Delaying the morning shift start time by 1h (7 a.m. instead of 6 a.m.) increased sleep duration by ~30 min.[153] This is a quite interesting finding, as people may not use the ‘extra time’ of a delayed start time for ‘extra sleep’. Furthermore, some studies reported longer sleep after the night shifts in permanent night workers as compared with rotational shift

workers.[154,155] Yet, when sleep duration was averaged across the entire shift cycle, they seemed to sleep somewhat less than workers in rotational schedules.[156] Besides schedule characteristics, inter-individual differences have been proposed to influence tolerance to shift work. Shift work tolerance can be operationalised as "the absence of problems commonly associated with shift work, such as digestive troubles, persisting fatigue and sleep alterations".[157,158] Accordingly, individuals showing longer sleep duration, better sleep quality and less circadian disruption are likely to be more tolerant to shift work.

1.4.3 Individual Differences in Shift Work Tolerance

On one hand, several studies have reported that eveningness might foster tolerance to shift work in general and night shifts in particular, due to higher quality and extended length of daytime sleep,[159,160] less rigid sleep-wake behaviour[161] and a later circadian phase delaying the fall of biological functions at night.[162] On the other hand, several reviews have questioned the predictive power of individual characteristics, such as chronotype, for shift work tolerance, mainly based on ambiguous results in that regard and the lack of longitudinal data.[158,161,163] However, some studies might have missed the very important fact that chronotype and type of shift interact on sleep-wake behaviour in shift workers.[164] Compared with early chronotypes, late types suffer more on morning shifts in terms of shorter sleep duration, worse sleep quality and wellbeing, as well as increased social jetlag as a measure for circadian misalignment (for social jetlag, see 1.3.5 and Ref.[3]). In contrast, early chronotypes have greatest difficulties on night shifts as compared with both, morning shifts and late types. Studies, not examining chronotype differences separately for each type of shift, are likely to over- or underestimate true effects, as these are simply outweighed. Additionally, assessing chronotype in shift work poses a challenge to most of the commonly used questionnaires possibly limiting their applicability. To address

this difficulty, the MCTQ[64] have been adapted to shift work settings, asking for the actual sleep-wake behaviour on each type of shift and the respective days off (Munich Chronotype Questionnaire for Shift-Workers, MCTQ^{Shift} [165]). Chronotype in shift workers can reliably and validly be determined via mid-sleep on free days after evening shifts (MSF^{E}_{sc}, corrected for a potential sleep-debt accumulated on shift days). Considering chronotype differences reveals huge potentials for the design of shift systems. Adapting schedules to individual temporal niches by reducing exposure to the most strenuous shifts (*i.e.* morning shift for late types, night shift for early ones) can increase sleep duration, improve quality of sleep and reduce circadian misalignment as was demonstrated by Vetter, Fischer, et al. in a recent intervention study that developed and implemented such a chronotype-adapted shift system in the field.[166]

1.5 Scope of Research

Circadian disruption, the mismatch of internal (*i.e.* chronotype) and external time (*i.e.* shift-associated working time), is one potential mechanism underlying shift work-associated diseases. Shift work involving circadian disruption was classified as probably carcinogenic to humans by the IARC in 2007.[130] Despite postulating circadian disruption as the causal link between shift work and diseases, the IARC did not provide a clear definition. The scarcity of theoretical frameworks as well as quantitative measures impedes the systematic investigation of its causes and consequences. Any quantification of circadian disruption, however, needs to take into account individual internal time, as otherwise true effects will be over- or underestimated.[167] This dissertation describes three projects studying circadian disruption in real shift workers. In **Project One**, the sleep-wake behaviour of 35 shift workers in a 12-h rotational schedule is examined using the MCTQ^{Shift} [165] and wrist-recorded actimetry data. The field study

demonstrates the importance of chronotype and shift sequence for circadian misalignment and sleep duration setting the groundwork for **Project Two**. A method to quantify circadian disruption of the sleep-wake cycle is proposed called ‘mid-sleep deviations’ that integrates two crucial aspects of sleep: internal time and sleep history. The measure uncovers a unique, distinct and chronotype-specific geometry of actimetry-based sleep-wake behaviour in 53 shift workers. Comparison with existent measures of circadian disruption confirms the validity of ‘mid-sleep deviations’ and highlights its additional information value. In **Project Three**, the proposed measure of ‘mid-sleep deviations’ is applied to evaluate sleep log data from 97 shift workers employed in seven different shift schedules. The results of mixed model analyses challenge current guidelines on night and shift work showing that the number of consecutive (night) shifts beneficial for an individual depends strongly on chronotype.

Each Project will be preceded by an introductory part, followed by a detailed description of the applied methods. Main results will be presented in the main text, and additional findings are provided in the respective Appendix enclosed at the end of each Project. The findings will be subsequently discussed in-depth and within the context of the current state of research.

2 Project One

Sleep-wake behaviour in a 12-h rotational schedule: The importance of chronotype and shift sequence

2.1 Brief summary

This study explores the sleep-wake behaviour of shift workers in a fast-forward rotational 12-h schedule, taking into account their individual chronotypes. Thirty-five participants at two plants at a German production site of BASF, a large-scale chemical company, were chronotyped using the Munich ChronoType Questionnaire for Shift-Workers ($MCTQ^{Shift}$) and wore actimeters for two consecutive weeks in the 12-h schedule involving rest periods of at least 24h after each shift (6 a.m. – 6 p.m., 24 hours off, 6 p.m. – 6 a.m., 48 hours off). Sleep and nap duration, social jetlag (a measure of circadian misalignment) and circadian phase markers (centre of gravity, mid-sleep) were computed from actimetry data. For night shifts, the earlier the employees' chronotype, the higher their social jetlag, the shorter they sleep, and the longer they nap (up to 3h). When main sleep and naps were added, early and late chronotypes slept approximately the same amount. Additionally, shift workers slept longest between day and night shift (~9h on average) which was more pronounced the later their chronotype. In the 12-h schedule, rest periods of 24h after a single day shift and 48h after a single night shift allow shift workers to immediately reduce their sleep debt after each shift. Via sleep-wake behaviour, the specific nature of the schedule may contribute to mitigating detrimental effects of shift work, as previously reported from employees working the schedule.

2.2 Introduction

Shift work has been associated with various adverse effects on health, sleep and safety,[1–3] and schedules involving 12-h shifts are no exception as evidenced by reports on obesity and high blood pressure,[4] irregularity of the menstrual cycle length,[5] sleep duration, vigilance and sleepiness,[6,7] to name only few examples. The major concerns against 12-h shifts have been reduced alertness and fatigue, which increase the risk of accidents thereby threatening both, safety and operational efficiency.[8] Yet, compressing the workweek into fewer days offers more leisure time, and therefore 12-h shifts are often popular with employees.[9] So far, research on the effects of extended working hours (comparing 12-h and 8-h shifts) produced ambiguous results.[10] For almost every outcome assessed (*e.g.*, alertness,[11–13] fatigue,[8,14,15] accident rates,[8,16,17] performance,[18–20] physical and psychological health,[21–23] sleep,[15,24] social life,[20,25,26] job satisfaction,[22,27,28] and sick leave and retention[29,30]) reported effects were negative, positive or neither. These contradictions can partly be attributed to different research designs, *e.g.,* studies assessing a change from 8-h to 12-h shifts where employees are highly satisfied with the new schedule and may not report adverse impacts.[9] Moreover, the implementation of a new schedule often involves changes not only in shift duration but also regarding the number of consecutive work and free days,[11] transition times,[24] direction,[31] and regularity,[22] challenging the interpretability and comparability of the findings.

Among all factors influencing tolerance to extended working hours, several studies have pointed out the importance of shift sequence.[13,32] Blocks of up to seven successive shifts promote the accumulation of sleep debt and fatigue,[6,7] which in turn increase health and accident risks of employees. Folkard and Lombardi proposed a “Risk Index” to estimate the injury risk associated with a work schedule and demonstrated that a schedule’s composition is more critical than the absolute weekly working time.[33,34] The overwhelming majority of schedules described in the literature involves at

least two consecutive shifts of the same type. In contrast, the schedule at the German manufacturing site of the large-scale chemical corporation BASF consists of one 12-h day shift, followed by 24h of free time, then one 12-h night shift with subsequently two days off. Previous studies found no differences between day work employees and shift workers in this schedule with respect to several outcomes. For example, shift workers did not show elevated injury rates, even after adjusting for job level (*i.e.* manual labor, supervisory) and type of job (*i.e.* production, maintenance).[35] Moreover, injury occurrences were consistently lower in the later hours of the shift whether working on day or night shift and irrespective of the employees' age. Oberlinner et al. observed a higher incidence of obesity, diabetes, and diseases of the circulatory and digestive system for shift workers as compared to day work employees.[36] However, this did not result in an increased risk of premature death, which was confirmed for cancer-specific mortality in a recent study.[37] Additionally, health status as assessed by the Work Ability Index[38] was comparable in shift and day workers.[39] Given that adverse effects of shift work are strongly linked to disturbed sleep and wakefulness,[40,41] the specific nature of the schedule may contribute to mitigating detrimental effects via sleep-wake behaviour.

As for sleep duration, the timing of sleep shows large inter-individual variation, reflecting differences in "chronotype".[42] Chronotype is defined as the phase of entrainment (Ψ), which represents the phase angle between the internal rhythm and the external zeitgeber cycle, *i.e.* between the sleep-wake rhythm and the light:dark cycle[43]. An individual's chronotype can be assessed with the help of the Munich Chronotype Questionnaire (MCTQ)[44]; specifically, mid-sleep on free days is calculated corrected for potential over-sleep (MSF_{sc}). Along with individual differences in chronotype, rhythms in melatonin and body temperature reach their peaks and troughs accordingly earlier or later.[45,46] The correlation between chronotype and internal rhythms also exists at the molecular level, *e.g.,* for *Per1*, *Per2* and *Rev-erba* expression profiles.[47]

Juda et al. showed that chronotype modulates sleep duration, sleep quality and social jetlag[48] (a measure for the mismatch between internal and external time) in shift workers.[49] While night shifts are especially strenuous for early chronotypes, late types are challenged by morning shifts. A similar chronotype-dependent pattern was most recently observed for napping behaviour in rotational schedules.[50]

Taking into account individual chronotype, actimetry-derived measures of sleep and circadian misalignment are analysed as well as circadian phase markers of both, the sleep-wake cycle and the activity-rest rhythm in order to examine sleep-wake behaviour in this 12-h schedule.

2.3 Methods

2.3.1 Study design and participants

The study took place in May 2013 at the BASF production site in Ludwigshafen, Germany, in two factories with similar chemical production processes and workplace requirements. Participants filled out the MCTQ^{Shift} [51] the first day of the study and subsequently wore actimetry devices for two consecutive weeks, comprising at maximum four day shifts, four night shifts and eight free days. Participants did not receive financial remuneration but were offered individual feedback about their chronotype and actimetry data. All participants gave their written, informed consent, and the local ethics committee as well as the BASF Works Council approved instruments and study protocol.

2.3.2 Shift Schedule

The schedule at the BASF production site was introduced in 1992 and relies on a fast-forward rotation with transition times at 6 a.m. and 6 p.m. Annual working hours are set to 1950h and overtime is balanced by additional time off. A 12-h day shift is followed the next day by a 12-h night shift and two

successive days off before returning to day shift equalling 24h off after day shift and 48h off after night shift.

Sleep sequence. The shift schedule comprises two workdays followed by two work-free days. Importantly, a different pattern emerges for sleep episodes linked to the schedule: employees start the shift sequence with sleep before the day shift, followed by a potentially unconstrained sleep episode (similar to a work-free day sleep) between day and night shift (starting at 6 p.m. the next day). After working the night, employees sleep during the day followed by a work-free day sleep before the cycle restarts with sleep before the day shift. The rest periods of at least 24h between each shift lead to an alternation of a single workday and a single free day sleep episode contrasting the shift schedule (see Figure 2.1 for comparison of work schedule with sequence of sleep episodes).

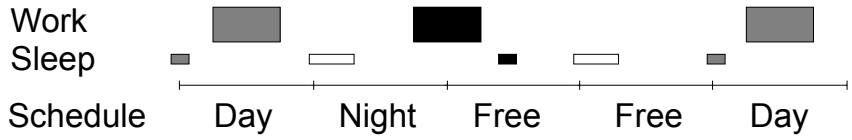

Figure 2.1. Shift schedule, working hours, and sleep. In the schedule, a single 12-h day shift is followed by a single 12-h night shift and subsequently two days off (Day – Night – Free – Free). The upper boxes indicate hours worked (grey = day shift, black = night shift), and the slim boxes beneath show the respective sleep episodes (grey = day shift sleep, black = night shift sleep, white = free day sleep). Since night shift starts at 18:00, the sleep episode between day and night shift is potentially unrestricted (*i.e.* no alarm clock), and therefore considered free day sleep.

2.3.3 Munich Chronotype Questionnaire for Shift-Workers (MCTQ^{Shift})

The MCTQ^{Shift} [51] has been adapted from the Munich ChronoType Questionnaire[44] to determine shift workers' chronotype in both, permanent and rotational schedules. It asks for simple information about the individual's sleep-wake behaviour, such as bedtime, estimated time to fall asleep, wake-up time, minutes to get up and use of alarm clocks, separately for each type of

shift and the respective free days. Mid-point of sleep on free days after evening shifts is used as a proxy for chronotype and corrected for potential sleep debt accumulated during shift days (MSF^{E}_{sc}). In case the shift schedule does not comprise free days after evening shifts, transformation algorithms were proposed to estimate MSF^{E}_{sc} from free days after morning and night shifts.

2.3.4 Actimetry

Wrist-activity was monitored using dual-axis accelerometers (Daqtix GbR, Uelzen, Germany) that record dynamic (motion) as well as static (gravity, *i.e.* change in position) acceleration. Data were sampled in 1s intervals and stored in 30s intervals. Subsequently, data were binned into 10min intervals. Participants could take off the device at any time and were asked to enter date and duration into a protocol. These times were later marked as missing data. Additionally, participants filled out a protocol to indicate shift days (day, night) and free days during the study period to account for irregularities in the schedule such as leave and sick days, overtime, etc.

2.3.5 Data processing and statistical analyses

Centre of gravity (CoG_{act})[52] /acrophase was calculated from actimetry data using the cosinor analysis based on a least squares approach that fits a one-harmonic cosine wave to a time-series for an activity-based marker of phase of entrainment (see [53] for equation).

Sleep on- and offset were determined from actimetry data applying a two-step method involving a predefined threshold and a bootstrap correlation analysis.[51,54] Based on these activity-derived sleep on- and offsets, mid-sleeps on work and free days (MSW, MSF), MSF^{E}_{sc} (chronotype proxy), main sleep duration, sleep debt, social jetlag[48] (a measure of circadian misalignment) and nap duration were computed. Sleep debt was determined

as the absolute difference of sleep duration between work and free days according to the formula below:

$$Sleep\ debt = \left| SD_f^S - SD_w^S \right| \tag{2.1}$$

where

SD^S_f = sleep duration of unrestricted sleep episode following shift "S" (*i.e.* between day and night shift, free day after night shift)
SD^S_w = sleep duration on workdays in shift "S" (*i.e.* day, night shift)

Social jetlag serves as a measure of circadian misalignment[48] and is calculated as the absolute hourly difference of mid-sleep on free and workdays:

$$Social\ jetlag = \left| MSF^S - MSW^S \right| \tag{2.2}$$

where

MSF^S = mid-sleep of unrestricted sleep episode following shift "S" (*i.e.* between day and night shift, free day after night shift)
MSW^S = mid-sleep on workdays in shift "S" (*i.e.* day, night shift)

To distinguish naps from main sleep episodes, a two-harmonic cosine fit analysis was conducted yielding daily cut-off values for time points below the activity-based MESOR (a rhythm-adjusted mean). Sleep episodes overlapping the range between both cut-offs were identified as main sleep, whereas sleep episodes outside this range were considered naps.

Statistical analyses were conducted with ChronoSapiens[54,55] and R[56] using the packages 'car'[57] and 'ppcor'.[58] Normal distribution was tested performing Shapiro-Wilk tests and normal Q-Q plots. In case of non-normality, non-parametric methods were applied such as (partial) Spearman's rho, Wilcoxon signed rank test and Mann-Whitney's U-test (which was conducted as a Wilcoxon rank sum test with continuity correction), indicated in parentheses. Additionally, an ordinary least square approach for regression line formulas was computed for validation of MCTQ^{Shift}-based chronotype with actimetry results. Plots of studentized residuals and fitted values as well as

the non-constant variance score test provided by the package 'car' assured homoscedasticity for linear regression modelling. Given the previously shown relationships between chronotype and age,[59] and age and sleep duration,[60] partial correlations were conducted with age and overall sleep duration as covariates for analysis of sleep-wake patterns. A partial correlation controls for other influencing parameters by removing their variation from both correlated variables. Overall sleep duration was calculated by the weighted arithmetic mean of daily sleep duration throughout the study period using the following equation:

$$Overall\ sleep\ duration = \frac{SD_w^S \cdot n_w^S + SD_f^S \cdot n_f^S}{n_w^S + n_f^S} \tag{2.3}$$

where

SD^S_w = sleep duration on workdays in shift "S" (*i.e.* day, night shift)
SD^S_f = sleep duration of unrestricted sleep episode following shift "S" (*i.e.* between day and night shift, free day after night shift)
n^S_w = number of workdays of shift "S"
n^S_f = number of unrestricted sleep episodes following shift "S"

Correcting for multiple comparisons in order to account for α – error inflation would have resulted in an increase of β – error probability and consequently in a decrease of statistical power (1-β). Given the relatively small sample size, a moderately conservative α-level of 0.025 is chosen and two-sided p–values are reported.

2.4 Results

2.4.1 Participants

Out of 39 initially participating shift workers two dropped out during the study period indicating personal reasons, and two were excluded *a posteriori* due to missing data. Demographic information on sex, age, body mass index (BMI) and chronotype of the remaining sample (n = 35) is shown below in Table 1.

Table 2.1. Demographic description of the study sample. yrs. = years, BMI = body mass index, MSF^{E}_{sc} = chronotype.

Characteristics	n (%) / mean ± std
Sex (% male)	33 (94.3)
Age (yrs.)	41.0 (±10.6)
BMI	26.9 (±4.2), range 20.1 – 35.8
MSF^{E}_{sc} (local time)	03:53 (±37min), range 02:59 – 06:08

2.4.2 Chronotype distribution/ $MCTQ^{Shift}$ evaluation

Chronotype (MSF^{E}_{sc}) was calculated from free days after night shifts applying the transformation algorithm proposed by Juda et al.[51] The $MCTQ^{Shift}$ results were compared with both, actimetry-based calculations of chronotype and CoG_{act} on free days after night shifts. Measures showed good congruency (Spearman's $r_{rho} = 0.55_{CoGact} - 0.70_{MSFEsc}$, $P < 0.001$; Fig. 2.2), as also indicated by the regression line formulas with slopes above 0.5 and intercepts close to zero. The sample's chronotype distribution was positively skewed with only two employees considered as late types[49] ($MSF^{E}_{sc} > 05:00$, German time) suggesting a slight advance compared to the expected normal distribution (Fig. 2.3). To test for this assumption, an age- and sex-matched sample was generated from the German database (www.theWeP.org, date: 10/17/2013) yielding an average of MSF_{sc} of (mean, local time) 04:06 (standard deviation) ± 32min. Comparing both populations revealed a significant advance of ~13min in the shift work sample (Wilcoxon signed rank test: $V = 176.5$, $P < 0.025$).

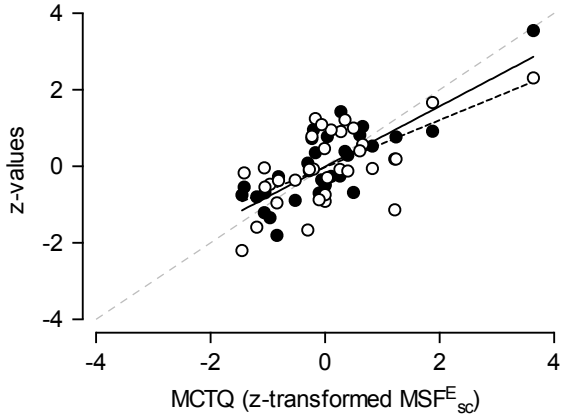

Figure 2.2. Evaluation of the MCTQ^{Shift} -based chronotype with actimetry measures (white dots = actimetry-based centre of gravity (CoG_{act}), black dots = actimetry-based MSF^{E}_{sc}). Grey dotted line represents the 1-to-1 diagonal. Values were z-transformed for direct comparison of slope and intercept of regression formulas. CoG_{act}: y = 0.62x – 0.0005; MSF^{E}_{sc}: y = 0.80x – 0.0012. MSF^{E}_{sc} = chronotype.

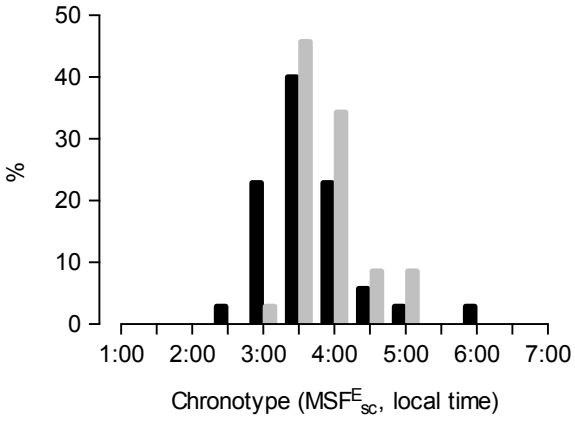

Figure 2.3. Chronotype distribution of the study sample and the matched sample from the database (www.theWeP.org). Black bars = study sample, grey bars = database. MSF^{E}_{sc} = chronotype.

2.4.3 Sleep duration

Across all shifts and free days, employees slept (mean ± standard deviation) 6h 49min ± 42min. Analysing the sleep episodes according to associated work and free days revealed shortest sleep duration before day shifts (5h 03min ± 53min) and ~30min more sleep after night shifts (5h 32min ± 59min). Sleep between day and night shift was longest with 9h 01min ± 82min, while employees slept approximately one hour less on free days after night shift (7h

49min ± 62min). Overall sleep duration was slightly higher the later the chronotype (r_{rho} = 0.27, P < 0.025).

Rank correlations with MSF^{E}_{sc} showed a chronotype-dependent pattern: the later an individual's chronotype, the longer sleep after night shift (r_{rho} = 0.41, P < 0.01). Sixty-nine percent (n = 24) slept longer on night than day shifts despite the comparably early chronotype distribution. No significant correlation was observed for chronotype and sleep duration before the day shift (r_{rho} = -0.07, P > 0.025). Yet, the later the chronotype, the higher sleep debt after day shift (r_{rho} = 0.37, P < 0.025) and the lower the night shift-associated sleep debt (r_{rho} = -0.37, P < 0.025; Fig. 2.4 A). Almost no sleep debt on night shifts (*i.e.* < 30min) was shown by four shift workers (11%).

When covariates age and overall sleep duration were included, partial correlations with chronotype were still significant for (i) sleep duration after night shifts (partial r_{rho} = 0.34, P < 0.025) and (ii) sleep debt after night shifts (partial r_{rho} = -0.38, P < 0.025). Yet, sleep debt after day shifts became independent of chronotype (partial r_{rho} = 0.26, P > 0.025); overall sleep duration, in contrast, accounted for 20% of the variance independent of age and chronotype (partial r_{rho} = 0.45, P < 0.01) suggesting that only shift workers sleeping longer in general showed greater sleep deprivation after the day shift.

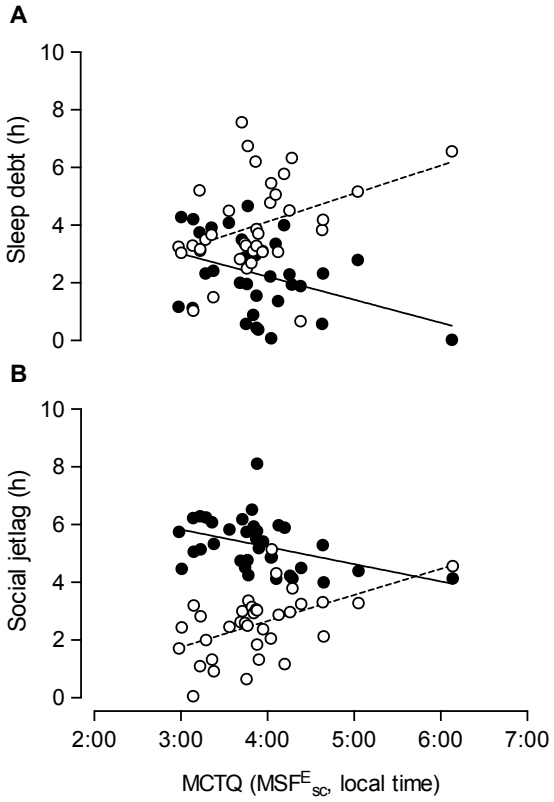

Figure 2.4. Chronotype-patterns of sleep debt (A) and social jetlag (B) on day shift (white dots) and night shift (black dots). Both measures are calculated as the absolute difference between work and free days, using sleep duration and mid-sleep, respectively. MSF^{E}_{sc} = chronotype.

2.4.4 Social jetlag

Social jetlag in the 12-h shift schedule was on average 3h 54min ± 31min, and was higher on night shifts than on day shifts (5h 18min ± 59min *vs.* 2h 33min ± 67min, Wilcoxon signed rank test: $V = 630$, $P < 0.001$). The relationship with chronotype was again independent of age and overall sleep duration: the later an individual's chronotype, the higher social jetlag on day shifts (partial $r_{rho} = 0.45$, $P < 0.01$) and the lower social jetlag on night shifts (partial $r_{rho} = -0.48$, $P < 0.01$; Fig. 2.4 B). Three out of 35 employees (9%) experienced less social jetlag on night than day shifts. None of the covariates age and overall sleep duration had significant influence (partial $r_{rho} = -0.25 – 0.28$, $P > 0.025$).

2.4.5 Naps

The majority of employees (n = 24, 68%) took naps before the night shift and 71% of them (n = 17) did so before each night shift, suggesting that taking naps was part of a personal routine. Nap duration was on average 1h 47min ± 29min with mean nap onsets and offsets at 13:19 ± 50min and 15:07 ± 43min, respectively. Time since main sleep offset was on average 5h 37min ± 65min. Converted to internal time (*i.e.* time since MSF^{E}_{sc}), employees napped in their subjective morning at 9:34 ± 49min after having a full night's sleep. Employees taking naps were earlier chronotypes (MSF^{E}_{sc} 3:44 ± 27min) compared with non-nappers (MSF^{E}_{sc} 4:11 ± 50min) (Mann-Whitney U-test: W = 195, $P < 0.025$). Furthermore, the earlier the chronotype, the longer they napped independent of age and overall sleep duration (partial $r_{rho} = -0.34$, $P = 0.10$; the coefficient reached significance when performing a sensitivity analysis by excluding the maximum (3h nap) and minimum value (47min nap): partial $r_{rho} = -0.50$, $P < 0.01$). When nap duration before night shifts was added to sleep duration after night shifts (resulting in a 24-h sleep duration), the previously observed correlation with chronotype disappeared (partial $r_{rho} = 0.05$, $P > 0.025$), as employees now slept approximately the same amount (Fig. 2.5). Yet, naps are often not distinctly relatable to one main sleep episode. Therefore, the 24-h sleep duration for the preceding main sleep (from day to night shift) was also calculated revealing the same pattern: rank correlations between chronotype and main sleep were significant (partial $r_{rho} = 0.31$, $P < 0.025$), but vanished for the nap-including 24-h sleep duration (partial $r_{rho} = 0.02$, $P > 0.025$).

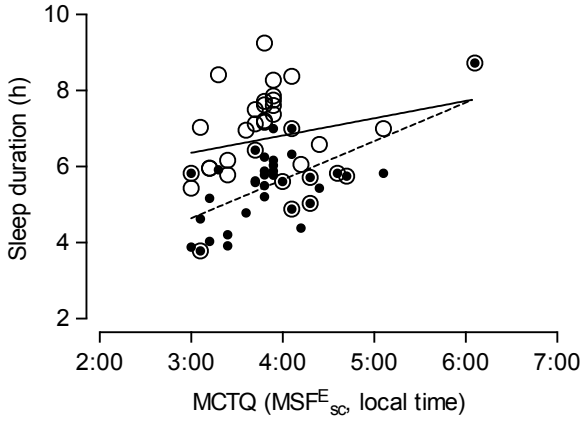

Figure 2.5. Naps and chronotype. The correlation of chronotype with main sleep after the night shift (black dots) statistically disappeared when nap duration before the night shift was added resulting in a 24-h sleep duration (open circles). Encircled data points represent participants *not* taking naps. MSF^{E}_{sc} = chronotype.

2.4.6 Continuous profiles of sleep and activity

Daily CoG_{act} serving as circadian phase markers for the activity-rest rhythm yielded a chronotype-dependent pattern throughout the study period: regardless of type of day (*i.e.* day shift, night shift, or free day), late chronotypes were always delayed compared with early and intermediate ones ($r_{rho} = 0.26_{day}$, 0.30_{night} and $0.51_{day\ to\ night}/0.55_{free\ day}$, $P < 0.025$) (Fig. 2.6). This finding clearly illustrates the link between chronotype and clock-regulated behaviour. Mid-sleep profiles revealed a similar pattern ($r_{rho} = 0.39_{night}$ $/0.64_{day\ to\ night}$ $/0.64_{free\ day}$, $P < 0.025$); however, no differences in sleep timing before day shifts were observed between employees ($r_{rho} = 0.15_{day}$, $P > 0.025$). Given that the same mid-sleeps can be reached by proportionally advanced and delayed sleep on- and offsets, respectively (*i.e.* a mid-sleep of 3:00 a.m. can result from sleeping between 1 a.m. and 5 a.m. as well as from sleeping between 11 p.m. and 7 a.m.), each sleep episode was analysed in more detail with regards to sleep onset and duration. All four sleep episodes were characterised by a distinct chronotype-pattern, that is visualised with plain symbols in Figure 2.7. Before the day shift, employees showed indeed no differences neither for sleep onset nor duration (partial $r_{rho} = -0.07_{duration}$ –

0.31_{onset}, P > 0.025). Regarding sleep between the day and night shift (a potentially unrestricted sleep episode resembling a work-free day), sleep onset was more delayed and sleep duration longer, the later the chronotype (partial $r_{rho} = 0.31_{duration} - 0.42_{onset}$, P < 0.025). After the night shift, all employees fell asleep at a similar time (partial $r_{rho} = 0.02$, P > 0.025) but the later their chronotype the longer they slept (partial $r_{rho} = 0.41$, P < 0.01). Finally, sleep onset on a free day after the night shift was again more delayed for later chronotypes (partial $r_{rho} = 0.59$, P < 0.01) but in contrast to sleep from day to night shift, sleep durations did not differ (partial $r_{rho} = 0.06$, P > 0.025).

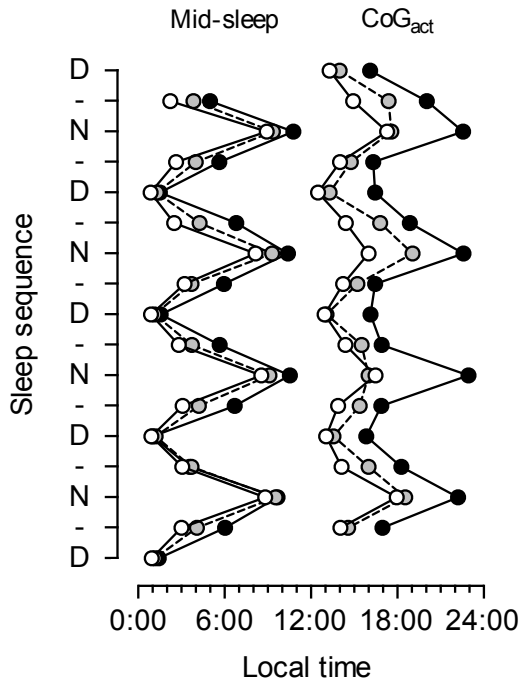

Figure 2.6. Continuous profiles of phase markers (centre of gravity, mid-sleep). White dots = early chronotypes, grey dots = intermediate types, black dots = late types. Cut-off values for early (<03:30, n = 10), intermediate (3:30≤ ≤05:00, n = 23) and late chronotypes (>05:00, n = 2) were arbitrarily chosen, but in accordance with previous studies.[51] Please note that all analyses were based on individual data points and categorical data are shown for illustrative purposes only.

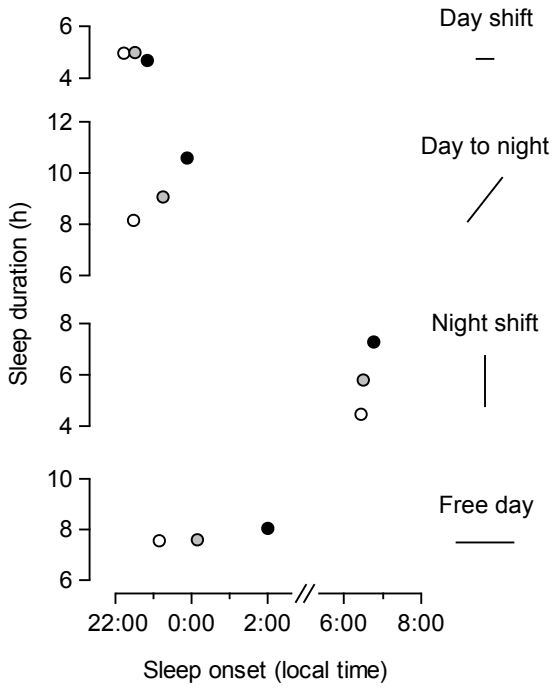

Figure 2.7. Chronotype-patterns of sleep episodes. Each of the four sleep episodes (before day shift, between day and night shift, after night shift, free day) is characterised by a distinct pattern visualised with the symbols aside. White dots = early chronotypes, grey dots = intermediate types, black dots = late types. Cut-off values for early (<03:30, n = 10), intermediate (3:30≤ ≤05:00, n = 23) and late chronotypes (>05:00, n = 2) were arbitrarily chosen, but in accordance with previous studies.[51] Please note that all analyses were based on individual data points and categorical data are shown for illustrative purposes only.

2.5 Discussion

This study examined sleep-wake behaviour of shift workers in a fast-forwards rotating 12-h schedule regarding their individual chronotype as assessed by the MCTQ^{Shift}.[51] In the 12-h schedule, single work and free day sleep episodes alternate frequently and fast, and thereby shift workers can immediately counteract sleep deprivation. Accordingly, the longest sleep duration (~9h) was observed between day and night shift, especially in late chronotypes. Two thirds of the employees took naps before the night shift, with early types napping both, more frequently and extensively up to 3h. When naps and main sleep were added for night shifts (24-h sleep duration), early and late types

slept approximately the same duration. The majority of shift workers showed a higher circadian misalignment (measured by social jetlag) on night shifts than on day shifts, comparable to workers in 8-h shift systems[49] but ~2 to 3h higher than in day workers.[61] Yet, most participants slept on average ~30min longer and had lower sleep debt after night shift compared with day shift. Additionally, it is noteworthy that despite the narrow range of chronotypes within the sample, a chronotype-dependent pattern was still observed with later types in general suffering less from the night shift than earlier types.

Continuous profiles of activity-derived circadian phase markers showed a chronotype-stagger with late types being consistently delayed compared with early and intermediate ones, except for day shifts where sleep timing was similar across chronotypes. This finding is in line with a previous study on daylight saving time showing that whereas mid-sleep fully adjusted after the spring transition, activity behaviour as indicated by CoG_{act} reached only partial or no adjustment dependent on chronotype.[62] Those results indicate that although sleep timing can somewhat accommodate external working times, activity rhythms do not, at least not to the same extent.

Partial correlations accounting for age and overall sleep duration showed a significant relationship between chronotype and sleep debt for night shifts but not for day shifts. On night shifts mainly early types were sleep deprived, while on day shifts chronotype did not account for sleep debt; only those sleeping longer in general showed sleep deprivation. The absence of a chronotype effect suggests that day shift started too early for all chronotypes available in the sample (range MSF^{E}_{sc} = 2:59 – 6:08). Although official transition times are 6:00 a.m. and 6:00 p.m., most participants indicated to be at their workplace half an hour earlier which corresponds to the observed ~30min of less sleep before day shift than after night shift. This is consistent with other studies reporting shorter or longer day shift sleep according to advanced[63] and delayed transition times,[7] respectively.

The extended sleep duration of ~9h between day and night shift agrees with previous reports suggesting that sleep between day and night shift is used for recovery rather than preparation.[63,64] The data presented here further support this assumption by contrasting two sleep episodes that are potentially unrestricted by alarm clocks, namely one on free days after night shift and the other from day to night shift. Only the latter one showed chronotype-dependent sleep durations, indicating catch-up sleep in late types given that day shifts are more strenuous for late than for early types.[49] However, early chronotypes also suffer more from night shifts than late ones, and consequently should show recovery sleep on free days after the night shift. Yet, when preceding naps and subsequent main sleep for night shifts were added, the resulting sleep durations were similar across employees. This suggests that extensive naps before night shifts may compensate for chronotype-related differences in catch-up sleep.

A study on sleep strategies in nurses working 12-h night shifts reported a similar pattern.[65] Nurses taking naps at work and those who were not, did not differ in their total sleep duration, but revealed different napping behaviours: either taking a long nap during the night shift followed by a short daytime sleep, or dispensing the nap at work but sleeping longer afterwards. In the present study, most employees took naps before the night shift, and since more than 70% of them did so before every night shift, napping appeared a personal routine; yet, early chronotypes napped more likely and extendedly. Given the preceding full night's sleep and the comparably early nap onset in the subjective morning only ~5h after main sleep offset, the regularity and duration of the observed naps is striking. Yet, external time of nap onset was around 1 p.m., assuming that participants potentially used a post-nutritional dip to fall asleep.

Naps were treated as being related to the night shift because participants indicated so in the MCTQ^{Shift}. However, time and rotational shift work are circular, and one could have also added the nap to the preceding

main sleep. In the present analyses, both approaches revealed the same correlative pattern suggesting that early chronotypes may nap to compensate a general sleep deficit.

The main disadvantage of 12-h shifts seems to be fatigue build-up over the period of work and its potential consequences for health, wellbeing, performance, and safety. Previous studies have argued that rest periods between changeovers may help to avoid excessive sleepiness and thereby improve sleep and sleep quality.[9,13] A study by Tucker et al. compared alertness levels, chronic fatigue and sleep in 12-h schedules with rest periods of 24h *vs.* 72h between day and night shift and found only modest differences.[66] They concluded that 24h off between day and night shifts might be sufficient to recuperate (given two preceding day shifts). Thus, providing at least 24h off between workdays seems to be a major advantage of this schedule. In general, recovery after work is strongly argued to be an explanatory mechanism in the relation between stressful work characteristics (such as long working hours) and health outcomes in the long run.[67] Adverse health effects of 12-h shifts may partially root in a chronic situation of sustained physiological activation (*i.e.* extended working hours, blocks of workdays) in combination with incomplete recovery (*e.g.,* short periods off work). Allowing for immediate reduction of sleep debt may therefore act as a contributor to the mitigation of adverse health effects in this schedule.

None of the reviewed studies described a rotational schedule with less than two consecutive shifts of the same type illustrating the uniqueness of the schedule. Only one study reported a permanent 12-h day or night shift schedule of health care workers with a single workday followed by 60h off.[68] The authors found no differences between day and night workers as to sleep complaints, which they explained by the nature of the schedule involving no successive night shifts. Yet, this result may have been confounded with the relatively low number of working hours per week (30h on average). Folkard and Lombardi demonstrated the importance of shift sequence for the relative

risk of accidents and injuries,[33,34] and a rest period of 24h between a single day and a single night shift may possibly add to the fact that injury rates were not increased in this schedule.[35]

Several studies have argued before that the sequence and the timing of shifts may be more important than the actual duration of the shift.[8,13] Ferguson and Dawson reviewed the question whether 'twelves' are better than 'eights' and postulate that in contrast to gender, age and domestic circumstances, shift pattern may impact directly on outcome measures.[10] Smith et al. examined the change from a slowly rotating 8-h schedule to a fast 12-h roster with one plant first implementing a rapid 8-h rotation (2x2x3) before permanently working 12-h shifts.[15] The 12-h schedule was superior to the slowly rotating 8-h system, while a within-plant comparison revealed no differences between the 12-h and the rapid 8-h schedule.

Several limitations of the present study are to mention. The chronotype range in the sample was quite narrow. Only two shift workers had a chronotype later than 5:00 a.m. which certainly impacted on the observed chronotype-dependent pattern; yet, based on previous literature,[49,50,61] the same and even more pronounced results would be expected with a wider distribution including chronotypes from both ends. Sleep-wake behaviour was assessed via activity measurements and the conclusions cannot essentially be broadened to other outcomes such as alertness and fatigue; further studies are warranted to elucidate those effects as well as synthesising research methods such as meta-regression[69] to examine the beneficial role of shift sequence on different outcome measures.

The present study emphasises the importance of both, shift sequence and chronotype for shift work research in general and studies on extended working hours in particular.

3 Project Two

Mid-sleep Deviation: quantifying and visualising circadian disruption of the sleep-wake cycle

3.1 Brief summary

Circadian disruption is argued to be a potential mechanism underlying the adverse health outcomes in shift work. Although the term is commonly used, only few definitions and even fewer measures exist impeding the systematic investigation of its causes and consequences. In view of a recent study demonstrating that mistimed sleep disrupts the circadian regulation of the human transcriptome, a novel and simple method is proposed to quantify the extent of mistimed sleep, called 'mid-sleep deviations'. Actimetry data over four weeks of 53 shift workers working in four different forwards-rotating schedules (55% female, age 35 ± 10 years, body mass index 26 ± 5) were analysed. Mid-points of sleep bouts were extracted daily and individual's chronotype was determined via sleep-wake behaviour on free days after the evening shift. The method takes into account two crucial aspects of sleep: internal time (*i.e.* chronotype) and sleep history (*i.e.* prior sleep episode). By eliminating the time dimension, a distinctive geometry emerges identifying differences across individuals, shifts, and schedules. Creating density plots to visualise the geometry, a higher variability of sleep timing was found the later the chronotype. This was independent of demographic variables, shift rotation, and sleep duration as confirmed by multiple regression models. Comparison with published measures of circadian disruption (*i.e.* inter-daily stability and 'behavioural entrainment') revealed good congruence; yet, analyses suggest that the concept of 'mid-sleep deviations' provides unique information on disrupted sleep-wake cycles. The less stable sleep-wake behaviour in late

chronotypes is argued to impact on development and prevention of diseases in shift workers and clinical patients, although its causes remain unclear. 'Mid-sleep deviations' as a measure for circadian disruption of the sleep-wake cycle will help to elucidate the role of mistimed sleep-wake rhythms in health on an individual basis.

3.2 Introduction

The endogenous circadian system actively synchronises (*i.e. entrains*) to the regular 24h alternation of light and darkness generated by the earth's axial rotation.[1] The external light messages are conveyed from melanopsin-expressing intrinsically photosensitive ganglion cells in the retina (ipRGCs) via a dedicated neuronal pathway to the suprachiasmatic nucleus (SCN) in the anterior hypothalamus.[2,3] The SCN is considered the master pacemaker creating an internal reflection of the external light-dark cycle thereby orchestrating biological functions over 24h.[4,5] At the molecular level, a complex machinery of transcriptional-translational feedback loops of gene expression[6] and post-translational regulation[7] generates stable circadian oscillations. The disruption of this fine-tuned time-keeping system is related to various adverse effects, such as insulin resistance and inflammation,[8] vascular events,[9] and disturbance of cell cycle checkpoints and DNA repair,[10] to name only few.

Through modern working requirements, such as rotational or permanent shift work, on-call duty, and overtime including weekends, internal clock-regulated rhythms are often in discrepancy to external social time which is referred to in many names, such as circadian disruption,[11] circadian misalignment,[12] circadian desynchrony[13] and chronodisruption.[14] The discrepancy between internal and external time (or as well among internal time-keeping systems) is especially pronounced in rotational shift workers and argued to be one potential mechanism underlying the well-documented health

problems.[15] These problems include obesity and diabetes[16], cardiovascular disease,[17] different forms of cancer (*i.e.* breast cancer,[18] prostate cancer[19]), sleep disorders,[20] and accident risk.[21] In 2007, the International Agency for Research on Cancer (IARC) declared shift work involving circadian disruption as probably carcinogenic to humans.[22] However, definition, assessment and quantification of circadian disruption vary from study to study, hampering consistent and systematic investigation of its causes and consequences.

The majority of studies define circadian disruption via its assessment resulting in a binary definition as 'present' or 'absent', and lacking a quantification of its extent. Assessments of circadian disruption in animal experiments include circadian time of carcinogen exposure,[23] inducing light shifts in jet lag protocols,[24] as well as maintaining constant conditions (*i.e.* constant light (LL)).[25] Other non-photic experimental manipulations of circadian disruption in animals involve ablation of the suprachiasmatic nuclei,[26] pinealectomy eliminating the secretion of the nocturnal hormone melatonin,[27] and use of mouse mutants with knocked-out clock genes.[28] In human epidemiological studies, circadian disruption is often assessed using proxies, such as shift work involving night shifts and 'light-at-night',[29] and occupations with frequent jet lags (*e.g.*, pilots and flight attendants with long haul flights).[30] In the laboratory, 'forced desynchrony' protocols are used to uncouple sleep-wake times from other circadian rhythms.[31] In those protocols, participants are placed, *i.e.* on a 28-h day under dim light conditions delaying their permitted sleep window by 4 hours each day while other circadian rhythms, such as body temperature and cortisol secretion, continue oscillating with a period slightly deviating from 24 hours. Thus, desynchrony of the internal circadian system can refer to misalignment with environmental time (*i.e.* jet lag) but also to desynchronization among internal timing systems (*i.e.* forced desynchrony) potentially resulting in aberrant temporal control of various physiological processes.

Despite their intuitive validity, most of the above-described assessments of circadian disruption lack both, clear theoretical framework and objective quantification. Few exceptions are found in the literature offering different approaches and definitions. Rüger and Scheer[11] define circadian misalignment as “mismatch of the circadian system with the desired sleep/wake cycle” (p.14) illustrating it as a continuum that ranges from extreme forms (*i.e.* blind people who are not entrained, shift work and jet lag) via more moderate examples (*i.e.* Advanced[32] or Delayed Sleep Phase Syndrome[33]) to mild ones (as seen in early and late chronotypes, or after staying up late while studying for an exam). This summarizes general situations and groups where circadian disruption can occur, and accordingly defines circadian disruption *post-hoc* via the presence of symptoms. Baron and Reid[12] basically distinguish between external (misalignment of the sleep/wake cycle in relation to the biological night, or misalignment of feeding rhythms to the sleep/wake or light/dark cycle) and internal misalignment (between central and peripheral rhythms). As potential causes they name chronotype, social jetlag, shift work, circadian rhythm disorders, disrupted feeding rhythm, and psychiatric disorders. It remains unclear whether some of the ‘potential causes’ may not actually be ‘potential consequences’ of circadian misalignment. Also, the concept of social jetlag[34] rather reflects than causes circadian disruption. Vetter et al.[35] build on the term ‘strain’ proposed in the context of stress research and define ‘circadian strain’ as individual response of a challenged circadian system. Depending on intensity and duration of the challenge, pathologies will or will not emerge. Thus, in this conceptual framework circadian strain does not necessarily result in adverse effects. Erren and co-workers defined the term ‘chronodisruption’[14,36,37] as ‘the adverse split of a physiological nexus of internal and external times’ (p. 291[37]) being a relevant disturbance of the circadian organisation of physiology, endocrinology, metabolism, and behaviour. They propose to evaluate gradients of exposure by calculating the extent of overlap between external

(*i.e.* shift start and end times) and internal time (*i.e.* chronotype). This conceptualisation would assume constant doses of chronodisruption irrespective of the number of consecutive shifts. Yet, it constitutes one of few approaches that attempted to not only define but also quantify circadian disruption.

Given the immense health burden by shift work[38] and its supposed causal relationship with circadian disruption[22], it is startling that –up to the author's knowledge- there is only a negligible number of quantifications reported in the literature. Burch et al.[39] sampled urinary 6-hydroxymelatonin sulfate (6-OHMS) in shift workers both, after work and after sleep, and evaluated the disruption of circadian melatonin production by calculating the sleep:work ratio of 6-OHMS concentrations. They found that risk for at least two symptoms of sleep disturbance, fatigue, and/or cognitive impairment was up to 8-fold in workers with ratios ≤ 1 and suggested that this ratio may help identify workers at increased risk for accidents or injuries. The inter-daily stability statistic[40] represents the 24-h value of the Sokolove-Bushell periodogram[41], normalised for the number of observations, and was used in multiple studies to assess fragmentation or disruption of activity-rest rhythms over time.[42–44] Rea et al.[45–47] suggested a measure for 'behavioural entrainment' using circular cross-correlations and phasor analysis for simultaneously recorded light and activity data. The resulting phasor magnitudes indicate how well activity-rest behaviour and actual light-dark exposure correspond showing that in humans and nocturnal rodents 'behavioural entrainment' is reduced when exposed to rotating shift work and jet lag protocols, respectively. Social jetlag, a concept proposed by the Roenneberg group[34] calculates the difference between sleep timing on work-free days and workdays. It reflects the impact of working times on sleep causing an accumulated sleep debt on workdays for most people that they eventually need to compensate for on their days off. The resulting shift in sleep-wake pattern resembles the one observed for time zone travels; yet, the

external light-dark cycle remains (almost) unchanged, thereby coining the term ‘social jetlag’. Despite being reliable and well suited for day workers, the calculation of social jetlag poses a challenge in rotational shift workers. Fast rotations, as common in Europe, involve different types of shifts (*i.e.* morning, evening, night) without interruptions by days off. Since the calculation of social jetlag relies on the difference between each shift and its respective work-free day, this leads to a potential over- or underestimation of social jetlag in shift workers. Yet, of all described approaches, social jetlag is the only measure quantifying disruption of sleep timing.

A recent study by Archer and colleagues[48] used a forced desynchrony protocol in humans demonstrating that sleeping out of phase with melatonin secretion reduced the number of circadian transcripts to 1% compared with 6.4% when sleeping in phase. They estimated the separate contribution of sleep and circadian rhythmicity and found that circadian-driven transcripts were associated with cellular metabolic and homeostatic processes, whereas transcripts driven by sleep alone (or by both, circadian rhythmicity and the sleep–wake cycle) were linked with the regulation of transcription and translation in particular. Importantly, melatonin profiles remained largely unaffected by mistimed sleep whereas the temporal organisation of clock gene expression, and therefore the molecular processes at the core of endogenous circadian rhythm generation, were altered. Thus, sleeping at the ‘wrong’ internal time, as occurs in shift work, might have tremendous impacts on health and wellbeing despite no effects on the central circadian clock as indexed by melatonin.

Here, a novel and simple method is proposed to estimate the degree of mistimed sleep, what is termed, for the purposes of this Project, ‘circadian disruption of the sleep-wake cycle’. The method can be used with various data sources, such as actimetry, melatonin, and sleep logs. It is however not limited to a specific type of data and can be easily transferred to sleep-unrelated measures. Daily mid-sleeps were used to quantify how much sleep

timing deviates across time from (i) sleep on previous day, and (ii) internal time, *i.e.* chronotype. Although sleep times are influenced by both, a circadian and a homeostatic drive, the mid-point of sleep has been shown to be a good behavioural phase marker of the circadian clock being independent from sleep duration.[49] Mid-sleep on free days (in shift workers: free days after evening shifts) corrected for potential over-sleep (MSF_{sc}), has been used previously as a proxy for chronotype,[50] and can be calculated from actimetry and sleep log data, or using a questionnaire (*e.g.*, Munich Chronotype Questionnaire, MCTQ[51]; Munich Chronotype Questionnaire for Shift-Workers, $MCTQ^{Shift}$ [52]). Here, chronotype is defined as the phase of entrainment Ψ of an internal rhythm with an external zeitgeber (time giver) cycle; specifically, as the phase angle between sleep-wake rhythm and light:dark cycle based on its assessment.[53] Each internal rhythm (*e.g.*, hormone secretion, core body temperature, clock gene expression) may assume a different phase relationship with its respective zeitgeber cycle (*e.g.*, temperature, feeding). It is therefore crucial to assess chronotype with regards to the rhythm of interest. Furthermore, individuals may show different internal phase relationships, yet the peaks and troughs of melatonin, body temperature, and *Per1*, *Per2* and *Rev-erbα* expression profiles were shown to vary with chronotype.[54–57]

In this study, actimetry-based sleep-wake behaviour was analysed in 53 rotational shift workers calculating the extent of mistimed sleep on particular days (*i.e.* night shifts) as well as for the entire study period. It will be further shown that chronotype differences seem to go beyond timing of sleep with a higher variability of sleep-wake behaviour the later the chronotype potentially impacting on development and prevention of diseases.

3.3 Methods

3.3.1 Study design and participants

Actimetry data of shift workers were merged from three different studies. The first study took place from May 26 to June 30, 2008, at a production site of the global company Siemens in Cham, Germany (study site 1); the second study was conducted from September 15 to October 15, 2009, at a plant in Berlin, Germany, of the same company (study site 2); and data from the third study were gathered between January 30 and February 26, 2012, at a production site in Bochum, Germany, of the large-scale company ThyssenKrupp (study site 3). A total of 76 shift workers wore wrist-actimeters ($n_1 = 28$, $n_2 = 23$, $n_3 = 25$) for a period between two and four weeks. A-priori defined exclusion criteria (see below) were applied resulting in a final sample of 53 shift workers ($n_1 = 23$, $n_2 = 17$, $n_3 = 13$). At the three study sites, shift workers worked in four shift schedules since two different ones were implemented at the plant in Berlin (study site 2, $n_2 = 9$, $n_{2:\ 555} = 8$) leading to four (independent) subsamples according to schedule. All schedules were forwards rotating and involved 8-h shifts with standard transition times for morning (6 a.m. – 2 p.m.), evening (2 p.m. – 10 p.m.) and night shift (10 p.m. – 6 a.m.). Except for the 555-schedule at study site 2, one rotation cycle was completed after 4 weeks. Figure 3.1 shows all four shift schedules.

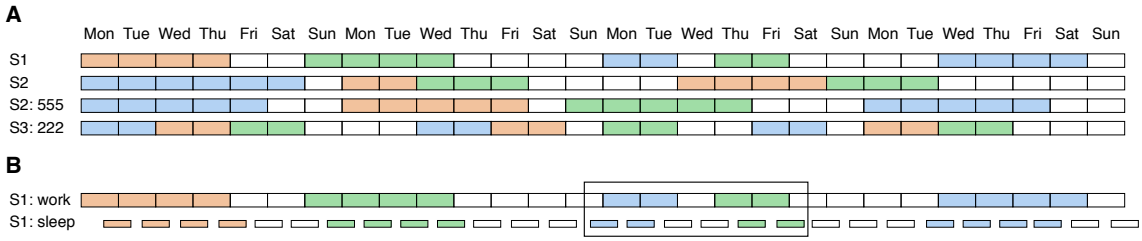

Figure 3.1. Shift schedules at the different study sites. **(A)** Schedule S1 refers to study site 1 in Cham, schedule S2 refers to study site 2 in Berlin (with two different schedules worked by two independent subsamples), and schedule S3 refers to study site 3 in Bochum (all study sites were in Germany). Each rotation is completed after four weeks, except for schedule S2: 555, which is completed after three weeks. Blue box = morning shift (6 a.m. – 2 p.m.), orange box = evening/afternoon shift (2 p.m. – 10 p.m.), green box = night shift (10 p.m. – 6 a.m.), white box = work-free day. **(B)** Using schedule S1 as an example, panel B illustrates how sleep episodes were assigned to (morning, evening, night) shifts. Sleep within rotational schedules might differ from sequence of workdays, *i.e.* sleep after the last night shift occurs in the morning of the first day off thereby compressing the number of 'free day' sleep episodes. The black-rimmed box marks an exemplary sequence: according to work schedule, two morning shifts are followed by one day off and two night shifts (S1: work); yet, sleep between a work-free day and the first night shift is considered 'free day' sleep, given the late start of the night shift allowing employees for sleeping in (S1: sleep). Likewise, sleep on first day off after a night shift takes place during the day and is associated with the night shift.

3.3.1.1 Exclusion criteria

Data of participants were excluded from analyses if (i) morning, evening and night shifts were present with less than two shifts of each type (n = 2), (ii) changes in work schedule (*i.e.* sick leave, vacation, overwork) exceeded the number of three shifts (n = 4), and (iii) data from more than three days were missing (n = 10). Apart from that, six participants dropped out during the study period indicating personal reasons and one had to be excluded due to a technical defect of the actimeter. An overview of the exclusion process can be found in the Appendix of this project (see Section 3.6.1, Table A3.1.).

Additionally, participants were excluded from regression analyses if their individual values were more than three inter-quartile ranges away from the sample mean (smallest sample size for regression models: n = 49).

3.3.2 Chronotype calculation

Chronotype was determined from actimetry data following the calculation by the Munich Chronotype Questionnaire for Shift-Workers ($MCTQ^{Shift\ 52}$). Mid-point of sleep on free days after evening shifts was computed corrected for a potential sleep debt accumulated on shift days (MSF^{E}_{sc}). In case the schedule did comprise less than two free days after evening shifts, chronotype was estimated from free days after night shifts applying the transformation algorithm proposed by Juda et al.[52]

The number of study instruments is often reduced to a minimum, and all variables needed for the calculation of mid-sleep deviation can generally be computed from one data source. However, it can sometimes be advisable to determine chronotype with the help of a questionnaire such as the MCTQ[51] or $MCTQ^{Shift}$,[52] allowing a general estimation over the last weeks. For example, if the study period does not include free days, or sleep times on free days are considered outlier values (*e.g.,* a social event keeping an early type up all night) impeding the reliable use of the data for chronotype calculation.

Although chronotype as measured via sleep-wake behaviour is a continuous biological trait,[53] it can sometimes be helpful to determine categories, representing early, intermediate and late types. Importantly, cut-off values for such categories should be either sample- or (if available) population-based because chronotype will depend on geographical location – amongst other factors–,[53] and a chronotype of 3:39 local time may be an early type in Central Europe but an intermediate type in India (relative to the population). Here, cut-off values for chronotype categories were calculated according to the sample's inter-quartile range, resulting in 13 early ($\leq Q_{25\%}$ = 3:53) and 13 late types ($\geq Q_{75\%}$ = 5:36). The terms 'early' and 'late' are therefore used in a correlative manner expressing differences relative to the sample. Please note, that all statistical analyses were based on individual measures and never on categorical data.

3.3.3 Actimetry

Dual-axis accelerometers (Daqtix GbR, Uelzen, Germany) were used recording dynamic (motion) as well as static (gravity, *i.e.* change in position) acceleration from wrist-activity. Data were sampled in 1s intervals and stored in 30s intervals. Subsequently, data were binned into 10min intervals for processing and analyses. If participants took off the device, they entered date and duration into a protocol, and these times were later marked as missing data.

To detect sleep from actimetry data, a two-step process was conducted that is described in more detail elsewhere.[58] First, the trend in the time series was calculated using a centred moving average within a 24-h window. A 20%-threshold of this trend was then applied to code values above ('wake') and below ('sleep') threshold resulting in new binary time series. Next, a 'cleaning'-procedure was performed on these new time series involving a bootstrap correlation analysis that generated multiple binary test series, and the one that correlated best with the uncleaned series defined the final sleep bout.

Additionally, only sleep bouts between 3h and 14h were included ignoring naps because the interest of this study pointed at what was considered main sleep. However, if a sleep bout was < 3h, it was included as main sleep if (i) it was the only one within 24h, or (ii) it had similar sleep onsets (night shift) or offsets (morning shift) as the previous sleep bout in a block of consecutive shifts. If two sleep bouts appeared within eight hours, only the first one was included. In case, a sleep bout shorter than 3h did not fulfil any of these criteria resulting in no sleep episode at all, sleep was imputed from available data to ensure continuous series of mid-sleeps.

3.3.3.1 Inter-daily stability and phasor magnitudes

For comparison of mid-sleep deviation with existing measures of circadian disruption, inter-daily stability[40] and phasor magnitudes[45] were calculated from actimetry data. Deriving from the Sokolove and Bushell χ^2-periodogram,[41]

inter-daily stability is computed from a dataset in the form of a “Buys-Ballot” table with K rows and P columns. Each row represents one day, and the number of columns is chosen according to bin size of data (*i.e.* 1h bins result in 24 columns, 10min bins in 144 columns). Each bin value and its column mean are compared with the overall mean of the table. Their ratio becomes maximal if bin value and column mean are identical, meaning that the time series on day i is exactly the same as on every following day. The empirical data series is thereby compared to a perfectly rhythmic time series indicating its extent of oscillatory stationarity.

Following another approach, circadian disruption is assessed in terms of ‘behavioural entrainment’.[45] Here, activity and light measures are compared using circular cross-correlations, and phasors are calculated via the resulting ‘behavioural entrainment’-correlation functions. A phasor is a rotating vector representing the complex constant $Ae^{i\theta}$ of a sinusoidal function $y(t) = A\ sin(\omega t + \theta)$ encoding amplitude and phase of an oscillation. The magnitude of a phasor is used to indicate the degree of ‘behavioural entrainment’ reflecting how strongly light levels and activity data are related. Fitting the sinusoidal curve over the entire study period was not meaningful resulting in too little between-individual variance. Thus, for the purposes of this study, the approach by Rea et al.[45] is followed calculating phasor magnitudes for each individual over the period of seven days including at least two night shifts, while inter-daily stability was computed for the complete study period in order to use all available information. Both measures were then compared with the degree of mid-sleep deviations for the matching period.

3.3.4 Imputation

Continuous time series are needed for the computation of mid-sleep deviations, and missing values (*i.e.* daily mid-sleeps) were imputed with average values from available data. Missing values could occur for two reasons: either no sleep bout was detected following the procedures

described above, or it was marked as missing data in the participant's protocol. Average values were calculated from matching sleep bouts, *e.g.,* if mid-sleep after a night shift was missing, the average was calculated from all but at least two night shifts available in the individual record. In total, 37 of 1464 observations (2.5%) across 22 shift workers (41.5%) were imputed with a maximum of three missing values per person. A descriptive comparison of the original with the imputed dataset is shown in the Appendix of this project (see Section 3.6.2, Figure A3.1).

3.3.5 Data analyses and processing

Sleep detection from actimetry was performed in ChronoSapiens[58,59] [Version 8.2]. Data processing, imputation and statistical analyses were done in STATA (Stata/SE 12.0), and plots were drawn in Prism (GraphPad Software 6.0) and R[60] using the packages "3dscatterplot" and "MASS".[61] Bivariate Pearson correlations were conducted (non-parametric rank correlations were computed in case of non-normality as indicated by Shapiro-Wilk tests) and multiple regression models controlling for schedule (with fastest-rotating schedule S3 as reference), age, gender (with female gender as reference), body mass index as well as number of children living in the same household (dichotomous factor with categories 0 = no children and 1 = one or more children). To meet the requirements for linear regression, Breusch-Pagan tests for homoscedasticity, Shapiro-Wilk tests for normal distribution of residuals, and calculated variance inflation factors (VIF) for multicollinearity were conducted. To account for multiple testing, Bonferroni-correction was applied resulting in an α-level of 0.006.

To create density plots, the R commands `kde2d` from the package 'MASS' for kernel density estimation and `contour` for contour line plots were used. Density plots consist of contour lines (also: isolines) connecting points where the function of two variables has the same value. Here, a two-dimensional kernel density estimation function was applied using default

bandwidths determined via normal reference distribution.[61] The number of grid points in each direction was chosen to be '200' forming a 200x200 – matrix for the density calculation in order to get smooth contour lines. Varying this number (*i.e.* 25 to 225 in steps à 50 grid points) did not result in a change of pattern. The following R code was used to create density plots:

```
#estimate kernel density
z <- kde2d(x, y, n=200, lims=c(-15,15,-15,15))

#create density plot
plot(y~x, xlim=c(-15,15), ylim=c(-15,15), xlab =
     expression(paste(Delta, " Chronotype")),
     ylab=expression(paste(Delta, " Day before")))

contour(z, nlevels=k, col=my.cols)
```

3.4 Results

The approach is exemplified with the individual data of one early (MSF^{E}_{sc} = 3:01) and one late chronotype (MSF^{E}_{sc} = 7:22) from shift schedule S1 but the data of the total sample (n = 53) is provided when illustrating an overall effect. For the individual examples, two shift workers who were as early and as late as available within the same working group were picked to ensure best-possible comparability between chronotypes. A demographic description of the sample can be found in the Appendix of this project (see Section 3.6.3, Table A3.2).

3.4.1 Mid-sleep Deviations: quantifying circadian disruption of sleep-wake behaviour

Daily mid-sleeps were extracted from actimetry data (see Section 3.3.3) and plotted over the course of the study period resulting in mid-sleep profiles (Fig. 3.2).

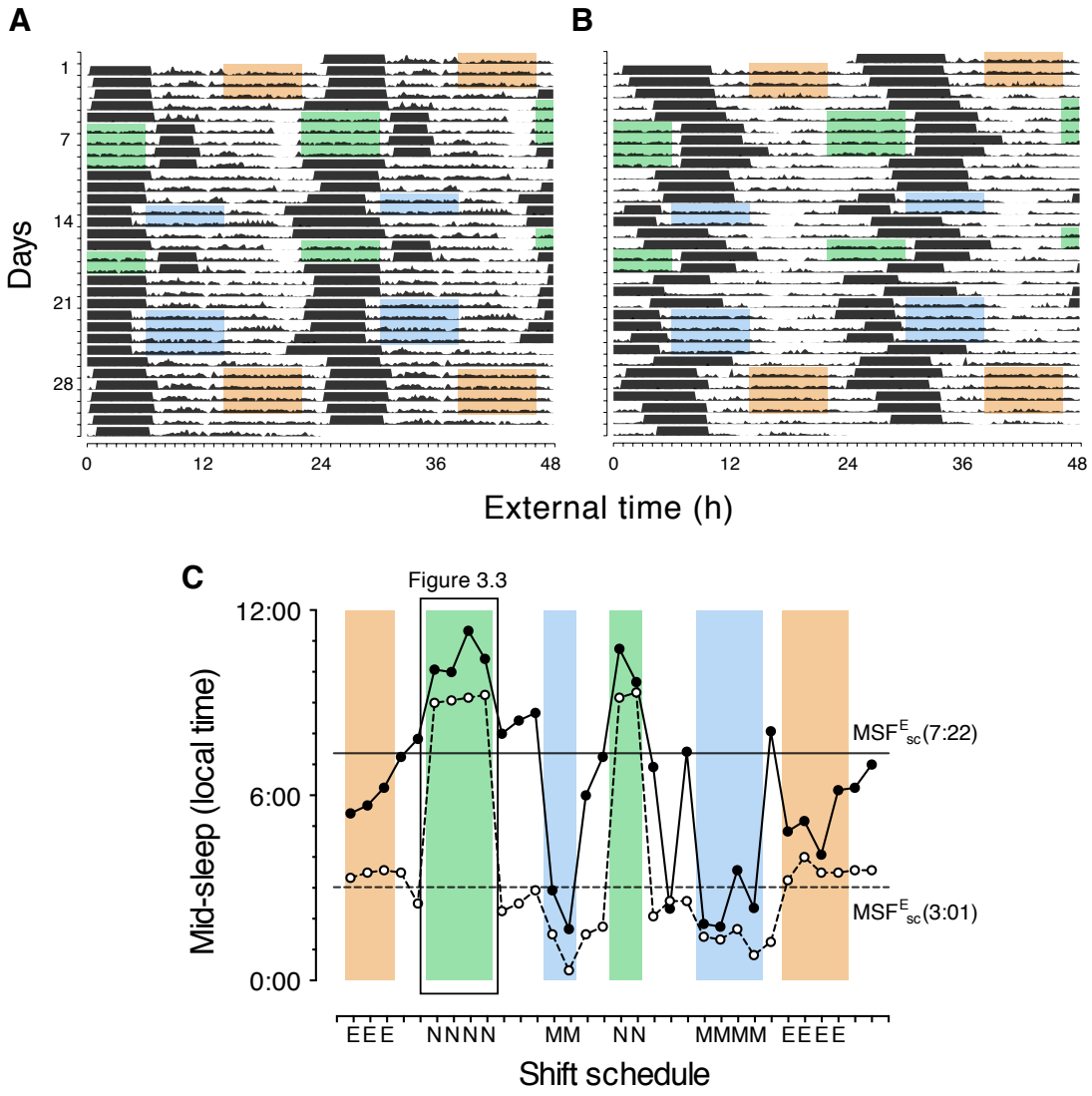

Figure 3.2. Double-plots of actimetry data over the course of 4 weeks of one early (MSF^{E}_{sc} = 3:01) (Panel **A**) and one late (MSF^{E}_{sc} = 7:22) (Panel **B**) chronotype from schedule S1. Double-plots show the same data twice to illustrate movements in activity and sleep patterns, *i.e.* first row displays day 1 and 2, second row day 2 and 3, and so forth. Black bars indicate sleep bouts. **(C)** Daily mid-sleeps extracted from

actimetry data of the early (white dots, dashed line) and the late type (black dots, straight line). Horizontal lines indicate individual chronotype (MSF^{E}_{sc}). Blue box = morning shift (6 a.m. – 2 p.m.), orange box = evening/afternoon shift (2 p.m. – 10 p.m.), green box = night shift (10 p.m. – 6 a.m.), white box = work-free day.

Two things can be observed: sleep timing accompanied external working times with large differences between morning and night shifts preventing shift workers from a stable sleep-wake rhythm; and, except for one day off towards the end of the study period, the late chronotype was always delayed compared with the early type, illustrating clear chronotype differences despite the same shift schedule. Thus, in order to quantify how much sleep times deviated, two variables were included: distance of sleep bout on day i from (i) individual chronotype (MSF^{E}_{sc}), and (ii) previous day i-1. Considering only one variable would result in over- or underestimation, because sleep times could move little over several days but be far away from chronotype (*i.e.* consecutive night shifts) (Fig. 3.3, lower panel), or they could move a lot but eventually match chronotype (*i.e.* free day after night shifts) (Fig. 3.3, upper panel).

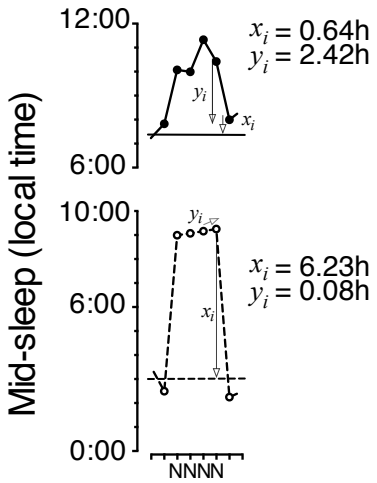

Figure 3.3. Sleep timing (daily mid-sleeps) on four consecutive shifts in the early (upper panel) and the late type (lower panel). x_i describes the difference between mid-sleep on day i and chronotype (MSF^{E}_{sc}, horizontal lines), y_i indicates the change in mid-sleep relative to the previous day. The values on the right illustrate that using either x_i or y_i would bias results.

The two variables are calculated according to the following equations:

$$\Delta \text{ Chronotype: } x_i = MSF_{sc}^{E} - MS_i \quad \text{(E3.1)}$$

$$\Delta \text{ Day before: } y_i = MS_{i-1} - MS_i \quad \text{(E3.2)}$$

where

MSF^{E}_{sc} = chronotype (mid-sleep on free days after evening shifts, corrected for over-sleep)
MS_i = mid-sleep on day i
MS_{i-1} = mid-sleep on previous day

The equations account for the agreement in the circadian field that positive values represent phase advances and negative values indicate phase delays. Both variables are then plotted against each other, creating a three-dimensional plot with ‘Δ Chronotype’ on the abscissa, ‘Δ Day before’ on the ordinate, and time course on the third axis (Fig. 3.4 A). Eliminating the time dimension results in a so-called ‘delta plot’, a four-quadrant scheme visualizing the interaction of ‘Δ Chronotype’ and ‘Δ Day before’ in circular, mainly clockwise movements (due to forwards rotation) through the four quadrants (Fig. 3.4 B).

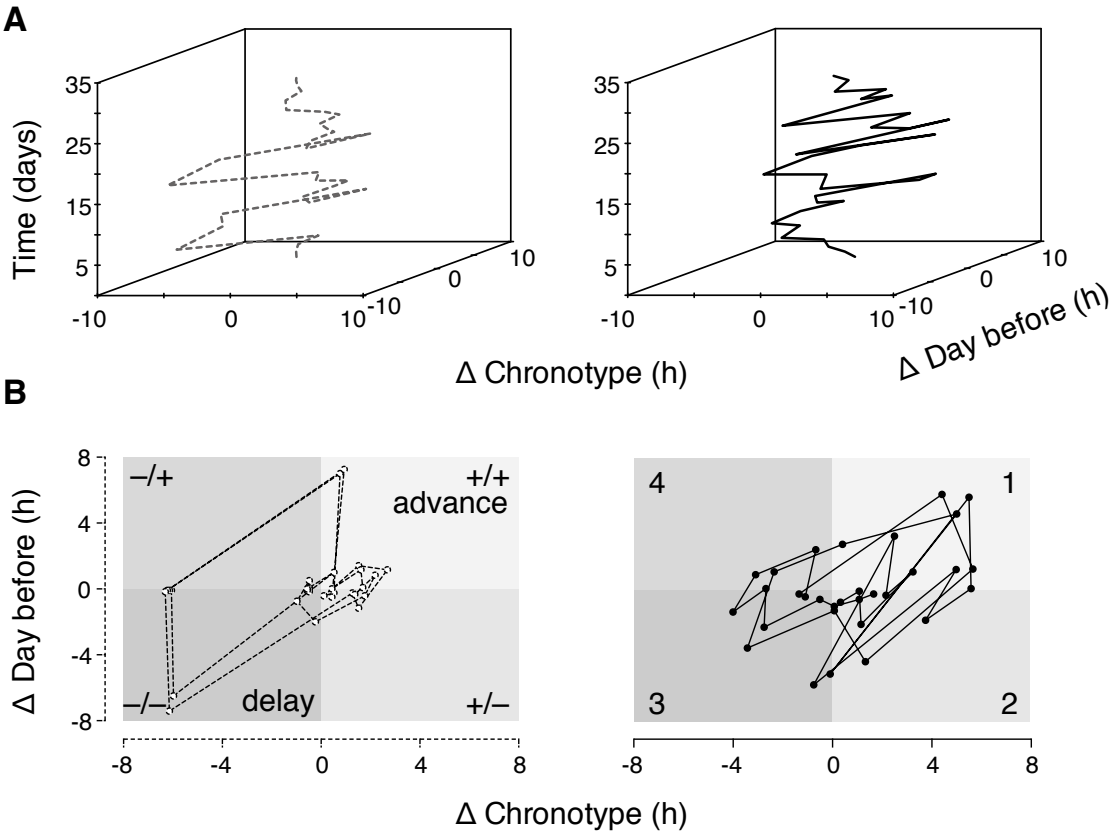

Figure 3.4. Three- **(A)** and two-dimensional **(B)** delta plots for the early (left panels) and the late type (right panels). The difference values x_i ('Δ Chronotype') and y_i ('Δ Day before') are plotted on abscissa and ordinate, respectively, leading to advances and delays throughout the shift rotation ('pure' shifts in quadrants 1 and 3, and 'hybrid' ones in quadrants 2 and 4). Panels B result from Panels A by eliminating the time dimension.

The deviation of any sleep bout is now quantified by determining the distance of its data point from the origin (Fig. 3.5). The distance is represented by the length of a two-dimensional vector and is calculated using Pythagoras' theorem:

$$\text{Mid-sleep deviation: } \left|\overrightarrow{MSD_i}\right| = \left|\begin{pmatrix} x_i \\ y_i \end{pmatrix}\right| = \sqrt{x_i^2 + y_i^2} \qquad \text{(E3.3)}$$

where

MSD_i = mid-sleep deviation on day i
x_i = distance of mid-sleep on day i from chronotype (MSF^E_{sc}, mid-sleep on free days after evening shifts, corrected for over-sleep)
y_i = distance of mid-sleep on day i to day before i -1

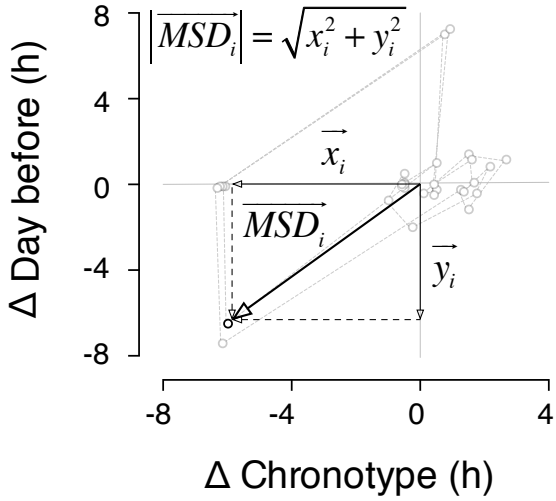

Figure 3.5. Calculation of mid-sleep deviation. The length of a two-dimensional vector calculated by Pythagoras' theorem indicates the distance of any data point to the origin.

Comparing the early and late chronotype with this measure immediately revealed differences according to the type of shift: the early chronotype showed largest deviations for night shifts, while highest values were observed on morning shifts for the late type (Fig. 3.6 A). Furthermore, there is a

continued impact of night shifts on the first day off for the early type, as well as for the late type after morning shifts, yet not to the same extent. Additionally, the early type showed either extreme high or very low values, whereas values of the late type were almost normally distributed (Fig. 3.6 B, upper panel).

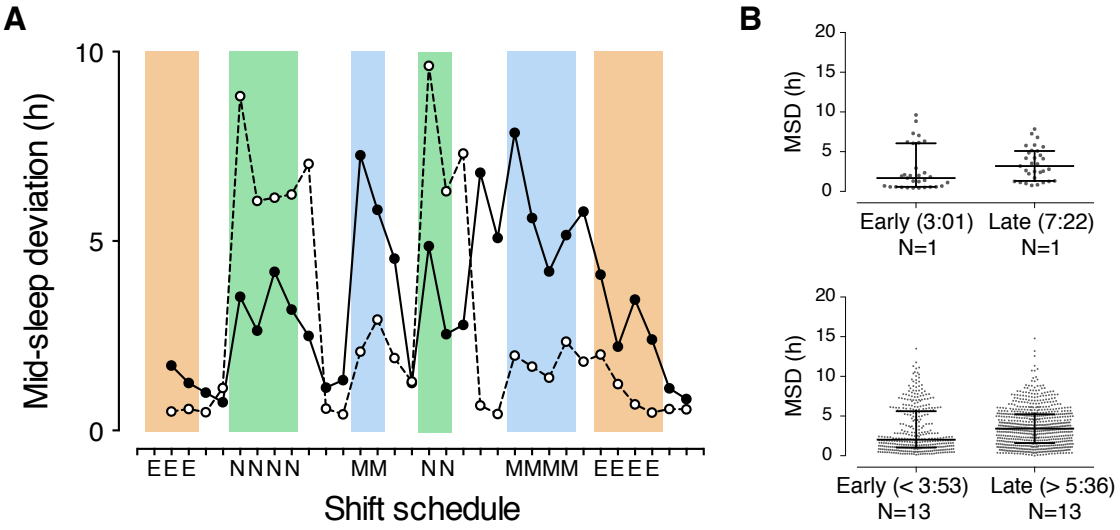

Figure 3.6. (A) Mid-sleep deviation values for the early (white dots, dashed line) and the late chronotype (black dots, straight line) throughout the work schedule. Blue box = morning shift (6 a.m. – 2 p.m.), orange box = evening/afternoon shift (2 p.m. – 10 p.m.), green box = night shift (10 p.m. – 6 a.m.), white box = work-free day. **(B)** Distribution of mid-sleep deviation (MSD) values in the two individuals (upper panel) as well as in all early (n = 13) and all late types (n = 13) (lower panel). Early types showed a positively skewed distribution, while values in late types were almost normally distributed.

Conducting regression models for the entire sample (n = 53) controlling for schedule, age, gender, body mass index and number of children revealed the same pattern: the earlier the chronotype, the higher deviations on night shifts and first subsequent day off ($\beta = -0.77_{night} / -0.53_{day\ off}$, $P < 0.001$), and the lower deviations on morning shifts and first respective free day ($\beta = 0.96_{morning} / 0.66_{day\ off}$, $P < 0.001$) (for full models see Appendix of this project, Section 3.6.4, Tables A3.3 – A3.6). Also, median was lower (2.04_{early} vs. 3.58_{late}) but inter-quartile range larger (4.52_{early} vs. 3.25_{late}) in early than late types,

confirming the pattern observed in the two individuals (Fig. 3.6 B, lower panel).

This procedure can be applied for both, particular days (*e.g.*, if one was interested in night shifts), and complete study periods (*e.g.*, if one wanted to compare work schedules). To integrate multiple vector lengths, one can use various modes according to data type and research setting (*i.e.* sum, maximum, average). Here, the mean was calculated to estimate the average effect of shift schedules on different chronotypes:

$$\text{Overall mid-sleep deviations: } MSD_{mean} = \sum_{i=1}^{n} \left| \overrightarrow{MSD_i} \right| \qquad \text{(E3.4)}$$

where

MSD_{mean} = averaged mid-sleep deviations over period n
MSD_i = mid-sleep deviation on day i

The regression model showed that each delaying hour of chronotype was associated with an increase of mid-sleep deviations by ~10min ($\beta = 0.46$, $P < 0.001$) (for full model see Appendix of this project, Section 3.6.4, Table A3.7). Additionally, a 'window of tolerance' was drawn based upon the relatively large discrepancy between sleep timing on work and free days that 33% of day workers experience.[62] The window included all values within a range of ±2h in both directions and the percentage of values outside this window was calculated (Fig. 3.7). Conducting a multiple regression revealed the same pattern as for the two individuals: the later the chronotype, the more data points were outside the window ($\beta = 0.75$, $P < 0.001$) (for full model see Appendix of this project, Section 3.6.4, Table A3.8).

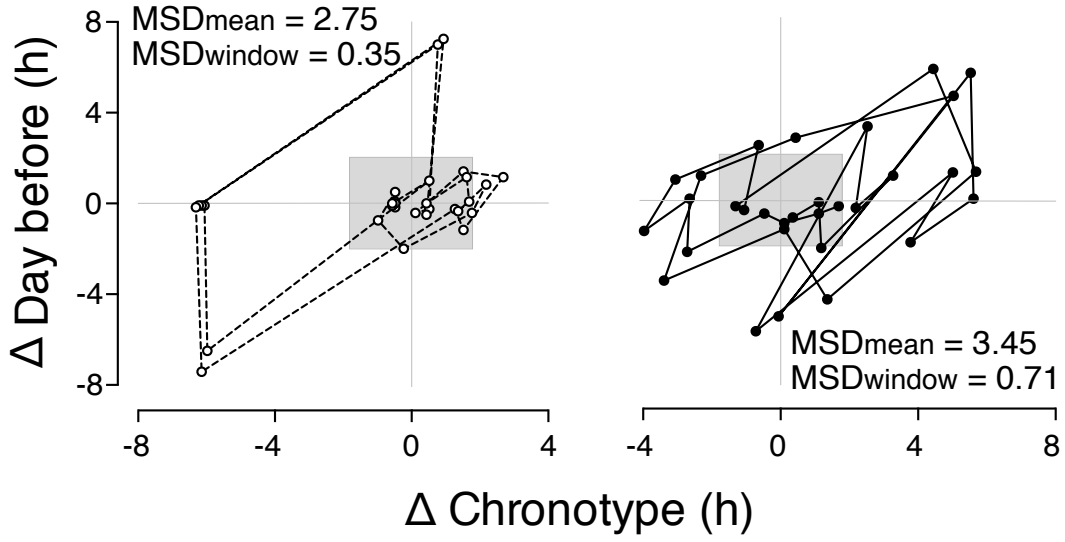

Figure 3.7. 'Window of tolerance'. The window covers all values within the range of ±2h in x – and y – direction for the early (left panel) and the late type (right panel). MSD_{mean} = mid-sleep deviations averaged across study period. MSD_{window} = percentage of values outside the 'window of tolerance'.

3.4.2 'Of islands and pancakes': sleep timing is more variable in late than early chronotypes

The delta plots revealed characteristic shapes, so-called 'chronotype-contours', that varied among early, intermediate and late types being differentially impacted by work shifts: early types appeared to be affected mainly (if not only) by the night shift resulting in large delays passing through quadrants 3 and 4 (Fig. 3.8, 1-13, blue box); intermediates seemed to be affected almost equally by morning and night shifts leading to a 'butterfly-shaped' pattern, moving trough all four quadrants (Fig. 3.8, 14-40); in contrast, late types showed greatest deviations by the morning shift (and less by the night shift) yielding large advances in quadrant 1 and 2 (Fig. 3.8, 41-53, green box).

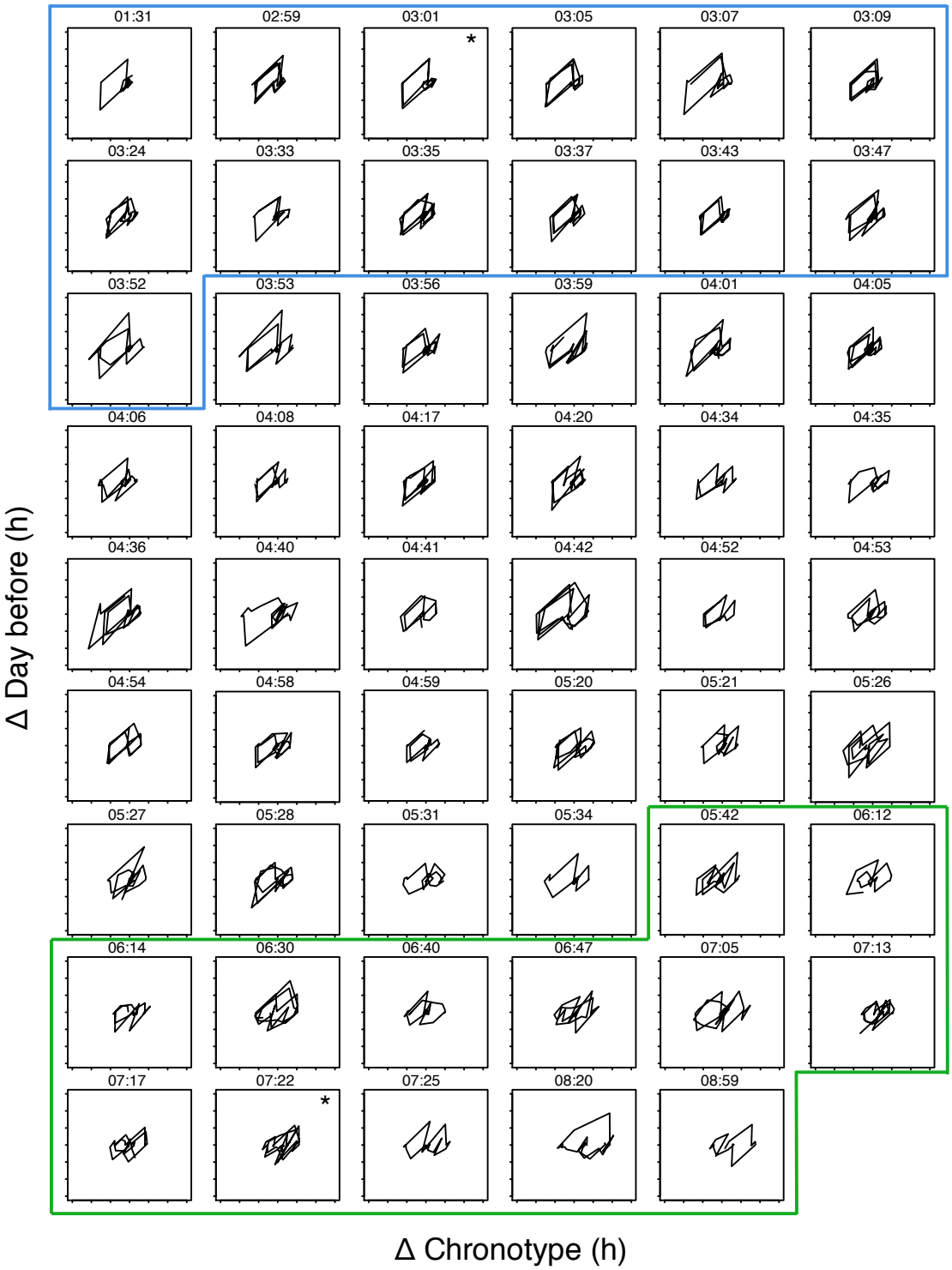

Figure 3.8. Delta plots according to chronotype. The blue box frames early types (n = 13, MSF^{E}_{sc} < 3:53), and the green box indicates late chronotypes within the sample (n = 13, MSF^{E}_{sc} > 5:36). The individual chronotype is shown above each panel as local time. Asterisks mark the two individuals used previously as examples for the approach. Axes range from -15 to 15, with a major ticks interval of 5.

Interestingly, there seem to be dissimilarities between early and late chronotypes that go beyond the differential patterns of delays and advances in sleep timing according to work schedule. A rotational shift worker is not expected to show stable sleep-wake behaviour in a circadian manner given the varying shift times. Nevertheless, a shift worker could show a (more or less) stable rhythm with a period of one rotation cycle, and if so, the lines in the delta plots should overlay indicating similar sleep times for equivalent shifts (*e.g.*, similar mid-sleeps for morning shifts throughout the schedule). Also, their days off should differ only marginally, at least if following the same type of workday (*e.g.*, similar mid-sleeps on free days following morning shifts), illustrated by a pile of data points near the origin. Thus, stability of sleep times can best be inferred by recurring shifts during the study period (as in the fast schedule S3 where employees work not only consecutive shifts but also several blocks of night shifts within one rotation cycle) showing that early chronotypes seemed more stationary (*i.e.* overlaying lines and data piles near origin) than late types being spread out within the quadrants.

To illustrate those differences, a simple visualisation was created of how dense the data of an individual were distributed, resulting in ‘density plots’ (Fig. 3.9). A density plot shows contour lines linking areas of equal density, similar to a topographic map where regions of the same altitude are connected with lines (for details, see Section 3.3.5). Here, the area covered by contour lines as an indicator for the extent of deviation, and the fragmentation of its shape as a marker for sleep time variance (*i.e.* a cohesive contour would suggest higher variance, as working times recur but sleep times differ widely leading to a ‘carpet’ of data points) are interpreted.

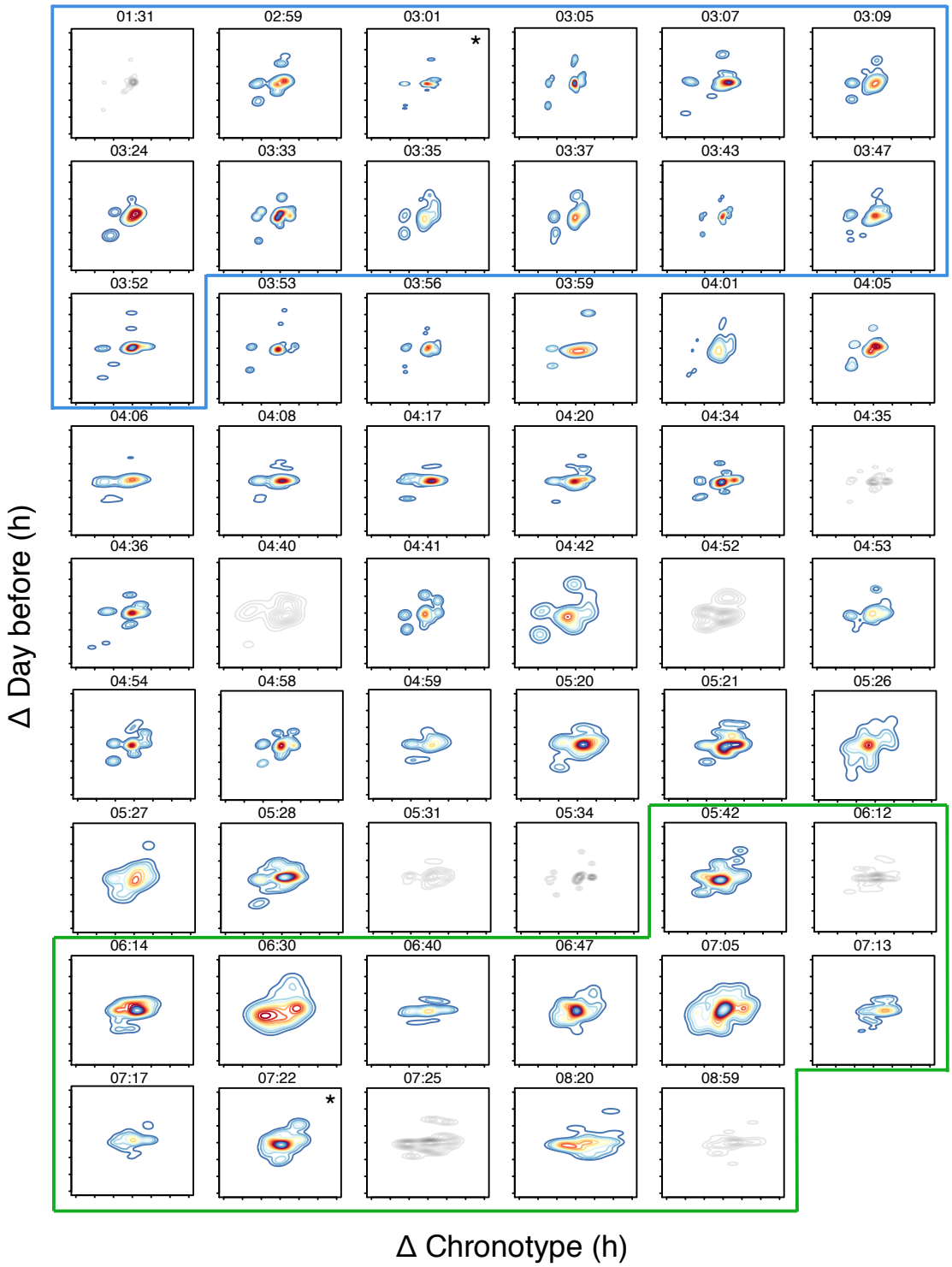

Figure 3.9. Density plots according to chronotype. The blue box frames early types (n = 13, MSF^{E}_{sc} < 3:53), and the green box indicates late chronotypes within the sample (n = 13, MSF^{E}_{sc} > 5:36). The individual chronotype is shown above each panel as local time. Asterisks mark the two individuals used previously as examples for the approach. Axes range from -15 to 15, with a major ticks interval of 5. Color code: dark blue = lowest density, dark red: highest density. Please note that data of

participants not working multiple blocks of (morning, night) shifts do not appropriately allow for inference of sleep-wake variability. Their data and density plots are not excluded but shown in light gray.

Inspection of density plots revealed remarkable differences between chronotypes: all early types consistently showed a fragmented pattern of 'data islands' that slowly moved across intermediate types towards a more unified pattern, eventually reaching cohesive contours in late chronotypes ('pancakes of data'). Figure 3.10 illustrates the differences in the extreme early and late types.

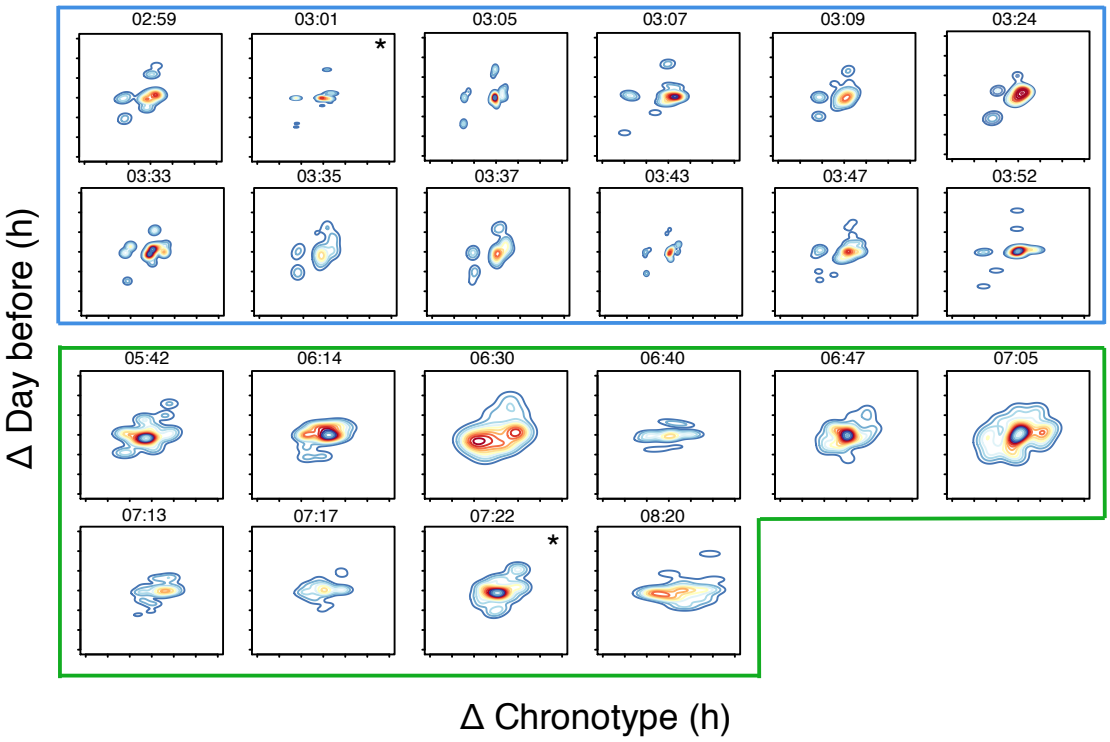

Figure 3.10. 'Of islands and pancakes'. Density plots of the earliest (blue box) and latest chronotypes (green box) in the sample. Fragmented patterns ('islands') as seen in the early types (green box) suggest more stable sleep-wake patterns as early types are mainly deviated by the night shift whereas sleep times of late chronotypes (blue box) are highly dispersed resulting in cohesive contours ('pancakes'). The individual chronotype is shown above each panel as local time. Asterisks mark the two individuals used previously as examples for the approach. Axes range from -15 to 15, with a major ticks interval of 5. Color code: dark blue = lowest density, dark red: highest density. Please note that data of participants not working multiple blocks of (morning, night) shifts were excluded only for the purposes of this graph.

In order to test this statistically, the ratio between median and inter-quartile range of mid-sleep deviations was calculated as a measure for how variable sleep timing was across the study period:

$$\text{Variability of mid-sleep deviations: } MSD_{\text{var}} = \frac{MSD_{median}}{IQR} \qquad \text{(E3.5)}$$

where

MSD_{mdian} = median of mid-sleep deviations over period n
IQR = inter-quartile range of mid-sleep deviations over period n

Please note that differential distributions of mid-sleep deviations were observed among early and late chronotypes as described previously (see Fig. 3.6 B). Early types showed few extreme values resulting in lower medians but larger inter-quartile ranges. This would have biased the use of inter-quartile range or other variance measures (*i.e.* standard deviation) as indicator for variability. Thus, the proposed formula accounts for positively skewed distributions as found in early types with lower values indicating lower variability. Accordingly, in case of normal distribution, no formula is needed and standard measures of variance can be used.

Regression analyses showed that variability was higher the later the chronotype ($\beta = 0.85$, $P < 0.001$) (for full model see Appendix of this project, Section 3.6.4, Table A3.9). Additionally, the same approach was calculated for sleep duration (overall sleep duration deviation, SDD_{mean}, and variability of sleep duration deviation, SDD_{var}) in order to see if different chronotype-contours were due to differences in sleep length (Fig. 3.11). No matching pattern was found, *i.e.* neither SDD_{mean} nor SDD_{var} were significant predictors of a more variable sleep timing as measured by MSD_{var} ($P > 0.05$); yet chronotype still explained 69% of the variance ($\beta = 0.83$, $P < 0.001$) (for full model see Appendix of this project, Section 3.6.3, Table A3.10). Furthermore, there was no relationship between chronotype and SDD_{mean} or SDD_{var}, (both r

= 0.01, P > 0.05) and none of the variables predicted overall mid-sleep deviation (MSD_{mean}) (P > 0.05, for full model see Appendix of this project, Section 3.6.4, Table A3.11).

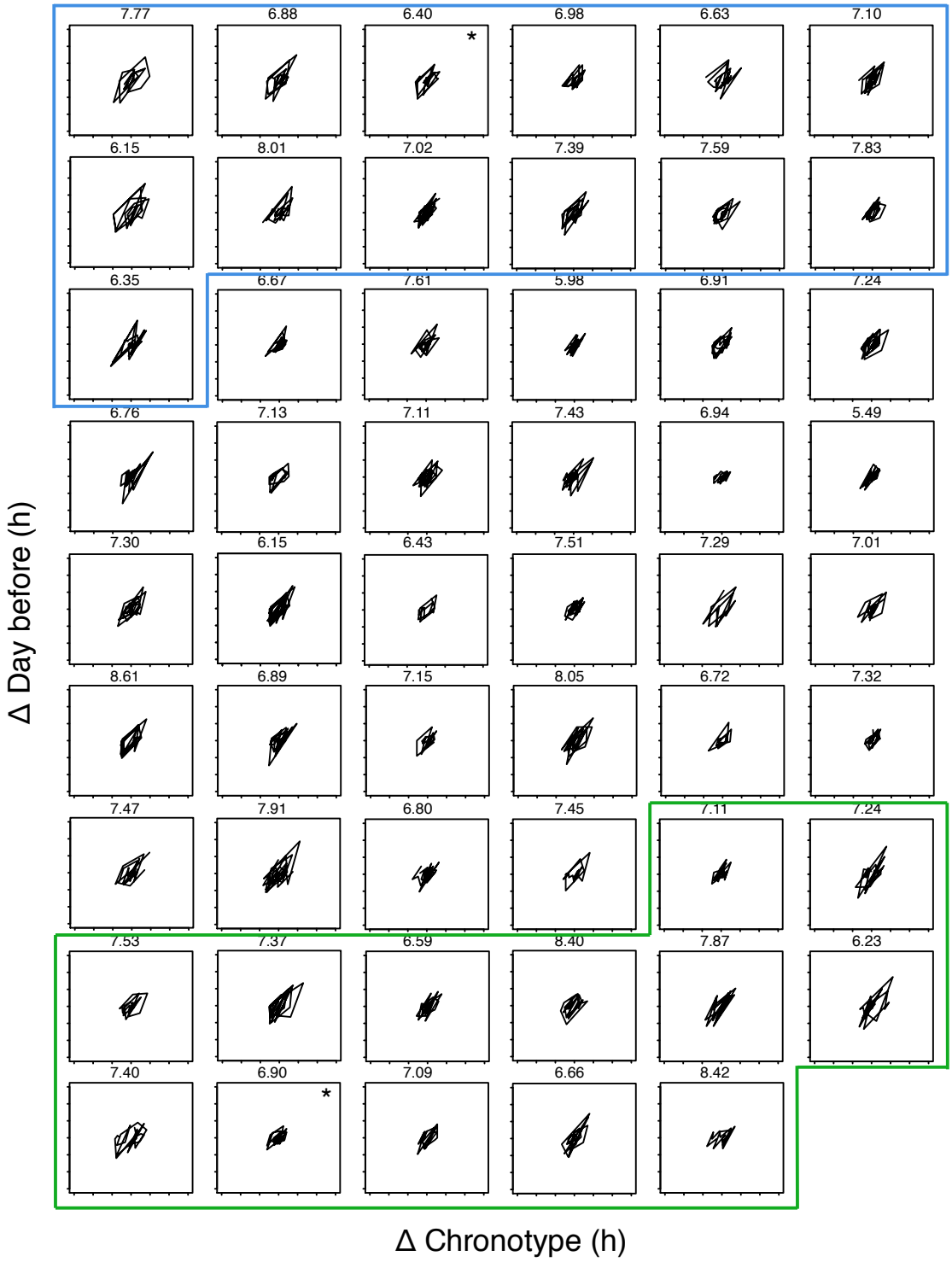

Figure 3.11. Delta plots for sleep duration according to chronotype. No pattern was observed that could explain chronotype-contours of mid-sleep delta plots, *i.e.* more or less variable sleep durations were similarly distributed within and between chronotype categories. The individual sleep duration (weighted average across the study period) is shown above each panel in hours. The blue box frames early types (n = 13, MSF^{E}_{sc} < 3:53), and the green box indicates late chronotypes within the sample (n = 13, MSF^{E}_{sc} > 5:36). Asterisks mark the two individuals used as prior examples for the approach. Axes range from -15 to 15, with a major ticks interval of 5.

Density plots are a fast and intuitive way of visualising differences across individuals. Moreover, they perfectly suit pre-post study designs. To illustrate this, additional data were analysed from participants in schedule S3 who had undergone the implementation of a new schedule that was adapted to the employees' chronotype distribution (details of this project are described elsewhere, see[63]). In the newly implemented schedule (*chronotype-adapted (CTA) schedule*), shift workers had been assigned to one of four groups according to their individual chronotype resulting in two 'extreme' shift groups comprising the earliest and the latest 25% of the distribution, respectively. Night shifts had been abolished for the early group and morning shifts for the late group. Their sleep-wake behaviour was assessed using actimetry and sleep logs once before the implementation and once right after it. Thus, two density plots were created for each individual allowing for direct comparison of pre- and post-measurement (Fig. 3.12). Just by the area covered, one can immediately see that the extent of mid-sleep deviations decreased in both groups and the 'islands' disappeared for the early types; yet, late chronotypes still showed expanded and cohesive contours ('pancakes') further supporting the notion of a higher variability in their sleep timing.

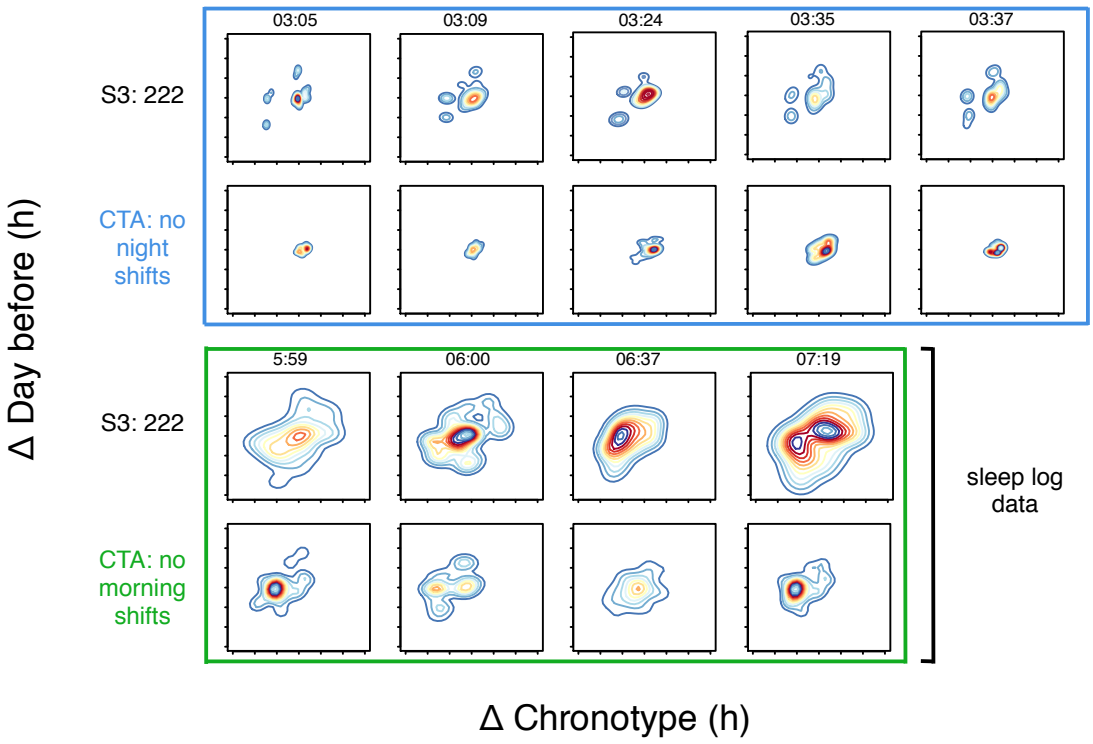

Figure 3.12. Density plots in a pre-post study design. Participants from schedule S3 took part in an intervention study where a chronotype-adapted (CTA) schedule was introduced abolishing night shifts for the earliest types (blue box) and morning shifts for the latest ones (green box).[63] They wore actimetry devices and filled out sleep logs before and directly after the implementation allowing for comparison of both schedules. Please note that no actimetry data were available from late chronotypes in the CTA schedule, and their sleep log data are shown instead. The individual chronotype is shown above each panel as local time. Axes range from -15 to 15, with a major ticks interval of 5. Color code: dark blue = lowest density, dark red: highest density.

3.4.3 Comparison with other measures of circadian disruption: 'inter-daily stability' and 'behavioural entrainment'

The concept of mid-sleep deviation was compared with two other measures claiming to quantify circadian disruption using actimetry measures (for details, see Section 3.3.3.1). The inter-daily stability[40] derives from the Sokolove and Bushell periodogram,[41] and quantifies the robustness of a circadian rhythm by the extent of oscillatory stationarity. 'Behavioural entrainment'[45] is quantified by the extent to which light exposure and activity data are related. Phasor

analyses of circular cross-correlations are conducted and the phasor's magnitude indicates how well light and activity measures match. First, overall mid-sleep deviations were correlated with inter-daily stability values and phasor magnitudes (Fig. 3.13 A). Both measures revealed a significant, negative relationship ($r = -0.56_{IS}$/ -0.48_{phasor}, $P < 0.001$) showing that higher mid-sleep deviations were associated with less robust activity rhythms and weaker light/activity-matching profiles (Fig. 3.13 A). As inter-daily stability and 'behavioural entrainment' are coded reversely (lower values signify higher circadian disruption), the negative value of the correlation reflects consistency between the measures.

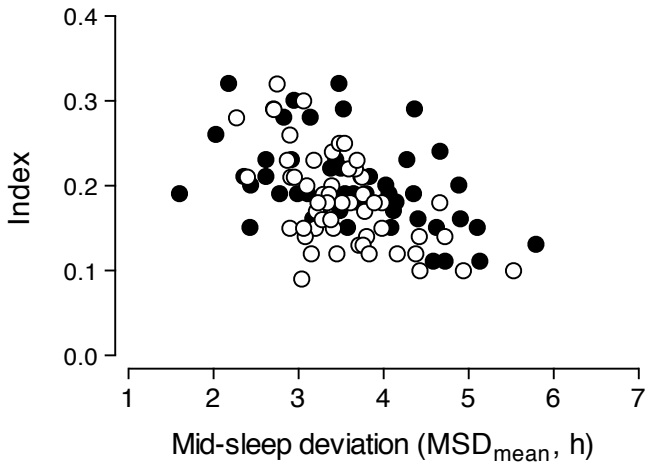

Figure 3.13. Comparison of mid-sleep deviations with inter-daily stability values (white dots) and phasor magnitudes (black dots). The negative relationships indicate congruence between measures, as inter-daily stability and phasors are coded reversely, *i.e.* lower values reflect higher circadian disruption.

Additionally, overall mid-sleep deviations (MSD_{mean}), inter-daily stability, and phasor magnitudes were correlated with the individual chronotype (MSF^{E}_{sc}) revealing good correlations for MSD_{mean} and inter-daily stability: the later the chronotype, the higher circadian disruption ($r = 0.37_{MSD}$/ -0.63_{IS}, $P < 0.001$) (Fig. 3.14). Yet, no significant relationship was found for chronotype and phasor magnitude ($r = 0.05$, $P > 0.05$) suggesting that two individuals (*i.e.*

opposite chronotypes) can largely differ in their day-to-day sleep-wake behaviour, but display similar degrees of 'behavioural entrainment' as long as they keep being active when lights are on and stop being active when lights are off. This finding is illustrated with two individuals from schedule S1 in Figure 3.15.

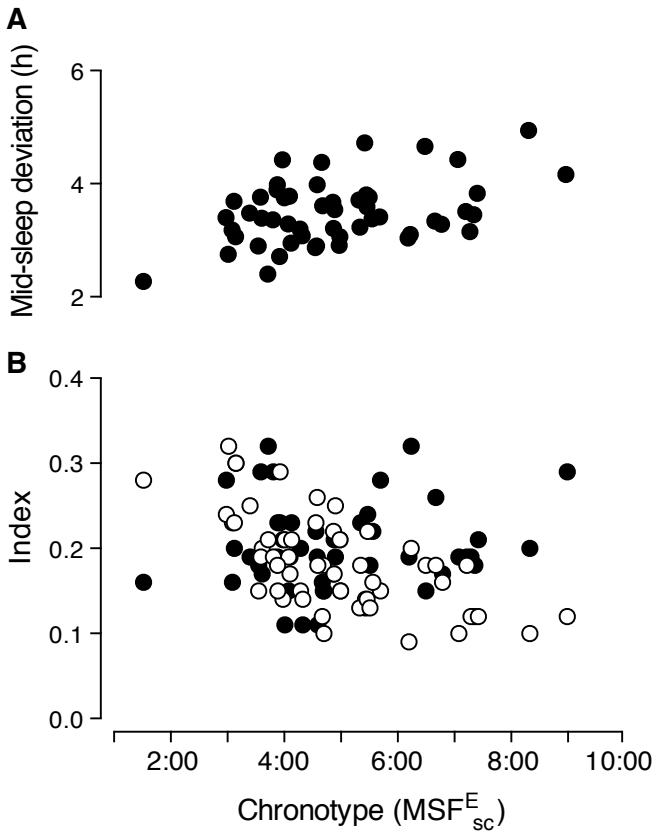

Figure 3.14. Relationship of measures for circadian disruption with chronotype (MSF^{E}_{sc}). Mid-sleep deviations **(A)** and inter-daily stability (**B**, white dots) showed good correlations, whereas phasor magnitudes did not relate to chronotype (**B**, black dots).

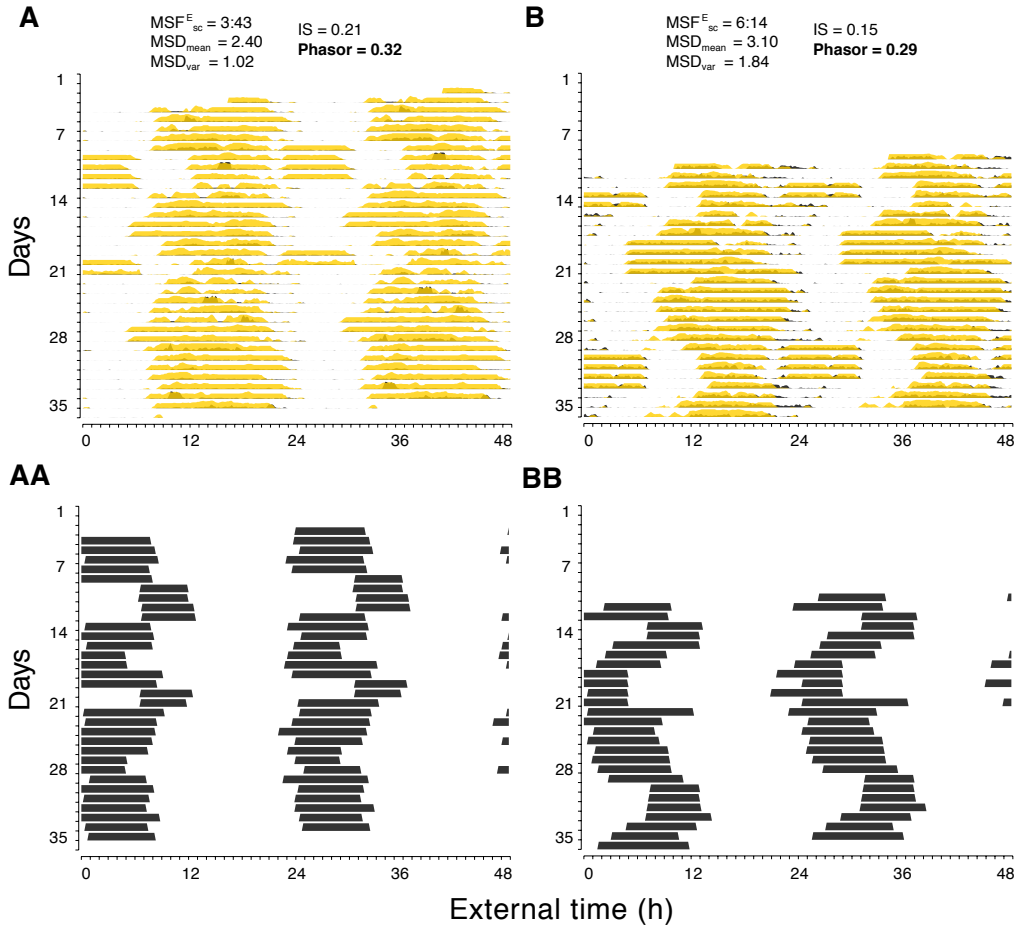

Figure 3.15. Double plots for light and activity (**A**, **B**), and sleep (**AA**, **BB**) in one early (left panels) and one late chronotype (right panels) from schedule S1. Phasor magnitudes are comparable between both participants indicating similar strength of 'behavioural entrainment'; yet, values for overall mid-sleep deviations (MSD_{mean}), variability of mid-sleep deviations (MSD_{var}), and inter-daily stability (IS) differ noticeably.

3.5 Discussion

3.5.1 Mid-sleep deviations as a measure for circadian disruption of sleep-wake behaviour

In view of an increasing number of people working in shifts, and adverse health consequences associated with shift work, researchers and practitioners are in need of a quantitative measure for systematically investigating the

assumed underlying mechanism, that is circadian disruption.[22] Here, a novel and simple approach is proposed to quantify circadian disruption of sleep-wake behaviour, called 'mid-sleep deviations'. The concept of mid-sleep deviations takes into account two crucial aspects of sleep in general: internal time (*i.e.* chronotype) and sleep history (*i.e.* previous sleep episode). Specifically, it calculates the distance of a particular mid-sleep from chronotype (using MSF^{E}_{SC} as proxy[52]) and previous mid-sleep. The resulting difference values are plotted against each other allowing for determining the extent of mid-sleep deviation by the length of a two-dimensional vector. It furthermore creates unique geometric patterns, named 'chronotype-contours'. The geometry of chronotype-contours can be visualised using density plots that calculate how dense the data are distributed resulting in coloured contour lines linking areas of similar density. The visualisation draws out chronotype-specific differences: while early types seem to display a quite stationary rhythm with similar sleep times on equivalent shifts, late chronotypes appear to have a higher variability showing more irregular sleep patterns.

Mid-sleep deviations can be (i) calculated from different data sources and types, such as actimetry, sleep logs, and biomarker levels, (ii) determined on single days (*i.e.* night shifts) and for entire study periods, and (iii) they are transferrable to various research settings, such as pre-post designs, cross-sectional studies, field and laboratory settings, and also sleep-unrelated scenarios. Another strength of this method is the easy-to-catch visualisation provided by density plots, perfectly suitable for pre-post study designs. It might help to identify health risk factors and effective interventions. It might also help to anticipate being at risk for diseases (*i.e.* relapsing episodes), particularly in psychiatric disorders where irregular sleep timing appears a symptom (*i.e.* bipolar disorder[64,65]). Additionally, it can be used for evaluation and comparison of shift schedules when companies plan to implement a new system. Although chronotype determined by mid-sleep on free days (after evening shifts) is part of the formalism, our approach is not limited to it.

Chronotype effectively represents a ‘baseline’ measure, and can be substituted by other variables depending on the data (*e.g.*, dim light melatonin onset on free days, averaged reaction times).

Mid-sleep deviations were compared with other measures of circadian disruption, namely inter-daily stability and ‘behavioural entrainment’. All measures showed good congruence indicating that although somewhat different in their approaches, they all reflect a shared phenomenon, at least to a certain extent. Yet, each measure has its pros and cons. The ‘behavioural entrainment’ concept relies on simultaneously measured activity and light data that can sometimes be difficult to assess in industry settings where devices are covered by working clothes (*i.e.* gloves) or not permitted at all due to safety restrictions. Furthermore, it does not reflect actual strength of entrainment (as the name suggests) but the extent to which light and activity data correspond. Let us consider two people sleeping in a room with open curtains. The person awakening earlier and being active earlier might not necessarily reflect stronger entrainment but simply an earlier phase relationship with the zeitgeber cycle. The term ‘behavioural entrainment’ should therefore be interpreted carefully. In contrast to mid-sleep deviations and inter-daily stability, phasor magnitudes were not related to chronotype. It is assumed that, *e.g.*, some early chronotypes might have had job tasks allowing them to largely reduce their activity during the nightly working hours matching the lower light levels and resulting in relatively strong ‘behavioural entrainment’ despite the night shift. Given similar mid-sleep deviations of those early types, the finding emphasises that, although correlated, sleep-wake rhythm and rest-activity cycle are not the same. In turn, lower magnitudes in late chronotypes might simply reflect a combined effect of their ‘habitually’ delayed activity patterns and generally lower light levels during night shifts (consequently resulting in an overlap of higher activity and lower light exposure). Thus, when using phasor analysis in an industry shift work setting, many late chronotypes will naturally display lower correspondence

between light exposure and activity behaviour. Yet, the approach is elegant and informative concerning the relationship between activity levels and actual light exposure, and combining both measures might further improve prediction of health risks.

The inter-daily stability is computed from activity measures, and applies an established algorithm to calculate how rhythmic daily activity-rest patterns occur. The basis of the algorithm is generally used to detect periodicity in time series, and derivatives have been proposed to indicate oscillatory stationarity of rhythms (*e.g.*, robustness index[66]). The usefulness of inter-daily stability has been demonstrated in various clinical settings with patients suffering from dementia where fragmentation of activity patterns frequently occurs.[42–44] However, by assuming a period of 24h, it implies a relationship with an external 24h-zeitgeber (*i.e.* daily alternation between day and night), whereas the *actual* zeitgeber (*i.e.* actual light exposure) might or might not have a period of 24h. Therefore, as Rea et al.[45] pointed out, relating activity-rest patterns to actual light-dark exposure might be the better assessment. Both measures, inter-daily stability and phasor magnitudes are limited to values between 0 and 1, and seem to work well for differences between day working and shift working participants, and between healthy controls and diseased patients, respectively; yet, they might be less useful to distinguish *within* a population of shift workers and patients given their limited variance. Accordingly, phasor analysis failed to detect differences in variability of sleep timing between chronotypes, as magnitudes will be high as long as activity and light levels match.

Although not assessed within this study, the 6-OHMS ratio proposed by Burch and co-workers will also be briefly discussed.[39] The 6-OHMS ratio requires sampling and analysis of urine, which is relatively costly and labour-intensive for both, researchers and participants. Melatonin is considered a robust and reliable clock output given neuronal projections between SCN and pineal gland.[67] Yet, melatonin might not be the best way to examine the

degree of circadian disruption, since it was shown to be relatively unaffected by timing of sleep despite a disrupted circadian regulation of the human transcriptome.[48] Probably the biggest strength of the concept of mid-sleep deviations consists in not being dependent on either melatonin or actimetry measures: the basic principle to quantify changes over time can be transferred to almost any kind of research setting and data. Yet, mid-sleep deviations rely on continuous times series. Imputation methods might help with this problem, but only to a limited number of missing values. Furthermore, fragmented sleep, several sleep episodes over 24h, or extensive naps probably contribute to sleep and health problems in a deteriorating or attenuating manner, and at least for now mid-sleep deviations cannot integrate several mid-sleeps on one day. Finally, similar successive mid-sleeps (*i.e.* on consecutive night shifts) will result in lower values by formula (*i.e.* decreasing 'Δ Day before'), which seems to contradict the recommendation for fast-rotating schedules in order to prevent accumulating fatigue and entrainment to the shifts.[68,69] However, fast rotations involve frequent shift changes, which in turn are associated to reduced longevity in animal experiments.[70] In general, mid-sleep deviations reflect known outcomes, such as early types having greatest problems with night shifts and continued impact of workdays on days off, and thus appear to be a promising approach to systematically examine physiological and psychological strain in different work schedules, clinical settings, and laboratory studies.

3.5.2 Higher circadian misalignment in late types

Analyses revealed that each delaying hour of chronotype was associated with a 10min increase in mid-sleep deviations indicating greater circadian disruption the later chronotype. There is a large body of studies investigating the role of chronotype and circadian misalignment for physiological and psychological health.[12] In evening types, studies found a 2.5 times higher likelihood to subjectively report poorer general health,[71] greater risk for

asthma,[72] a 2.5-fold increase in type II diabetes and a 1.3-fold increase in hypertension prevalence,[73] poorer glycaemic control in a sample of patients with type II diabetes,[74] greater risk for obesity,[75] greater body fat among a sample of participants with bipolar disorder,[76] and higher cigarette and alcohol consumption as well as lower physical activity.[77] Additionally, evening type was associated with higher cognitive ability but lower academic achievement.[78] With regards to psychiatric illnesses, multiple studies focused on development and severity of affective disorders (*i.e.* major depression,[79–81] seasonal affective disorder,[82] bipolar disorder[83,84]). These studies demonstrated that 'having' a late chronotype (usually assessed via MEQ) was associated with, *i.e.* higher depression scores, severer symptoms and shorter relapse intervals.

Most of the studies controlled for overall sleep duration, concluding that chronotype had an impact above and beyond sleep length. Yet, as shown by several studies, late types sleep less on workdays and sleep longer on weekends thereby compensating for a work-related sleep debt.[49,62,85] This results in similar overall sleep durations for early and late types studies have controlled for, despite differences in sleep on work and free days, as reflected by social jetlag. Social jetlag in turn has been associated with a higher likelihood of being a smoker, consuming alcohol and caffeinated beverages,[34] a 33% increase in risk of being obese with every hour of social jetlag (when already having weight problems),[62] and a higher risk for depressive symptoms[86] as well as cardiovascular problems.[87] These findings revisit previously reported results and raise the question if chronotype effects are rather mediated by circadian misalignment.

Accordingly, several studies proposed circadian misalignment as potential mechanism for physiological and psychological impairments, either arguing that eveningness itself is a form of circadian misalignment (*e.g.*, late types are reported to have shorter phase angles, and therefore sleep at an earlier circadian phase[55,57,88]) or suggesting a higher probability for late

chronotypes to experience misalignment. Yet, only few studies accounted for a measure of circadian disruption. One recent study included circadian misalignment assessed as self-reported habitual bedtime earlier or later than preferred bedtime, and found that both, late chronotype and 'misaligned' bedtimes were independent predictors of shorter disease-free intervals of metastatic breast cancer.[89] Levandovski and colleagues examined separate contributions of chronotype and social jetlag to depression.[86] They conducted rank correlations with depression scores assessed by Beck's depression inventory (BDI) within three categories of chronotype (early, intermediate, late) and social jetlag (≤2h, 2-4h, >4h), respectively, and found significant relationships for social jetlag and BDI in each chronotype group, but only one significant correlation for chronotype and BDI in the lowest social jetlag category. This finding suggests a greater role for social jetlag than chronotype in the prevalence of depression symptoms, but the analyses could not exclude chronotype as an independently contributing factor.

There are several, substantial problems to separate the unique contributions of chronotype and circadian misalignment in observational studies, and these aspects are symptomatic for most (epidemiological) research on chronotype and circadian misalignment. First, when deciding on artificial categories cut-off values are crucial, and if unfortunately chosen, results are biased in a way that might obscure true effects. Second, using continuous statistical methods (rather than mean comparisons) that control for the influence of a third variable (*i.e.* partial rank correlations) would more appropriately allow for elucidating the distinct contributions of social jetlag and chronotype to prevalence of diseases. Third, and most importantly, the fact that in the study by Levandovski et al. only 81 early and intermediate types experienced more than 2h of social jetlag whereas 296 late types did so, suggests such a strong relationship between chronotype and social jetlag, that statistical control of chronotype is no longer possible as it relies on sample values (meaning that if all variance in the moderate and high social jetlag

groups comes from late types, no reliable statement can be made for early and intermediate types). Accordingly, this is a general and essential problem when examining separate contributions of chronotype and circadian misalignment (*i.e.* social jetlag). If there are no early chronotypes suffering from a significant amount of social jetlag in the real world, it will never be possible to tell from observational studies that social jetlag (rather than chronotype) is the 'true' factor contributing to these disorders because only eveningness ever comes along with high social jetlag. Consequently, experimental studies would be needed clearly facing serious ethical issues. In the end, it is possible that social jetlag would indeed account for all 'chronotype-related' inter-individual variance of disorders, such as depression. Thus, adverse health effects in late types could be explained assuming socially induced sleep deprivation and circadian misalignment rather than being a late chronotype per se. Despite statistical challenges, including a measure for circadian misalignment, such as mid-sleep deviations, social jetlag, or 'behavioural entrainment', will always improve validity of predictions.

3.5.3 Higher variability of sleep-wake behaviour in late chronotypes

Numerous studies have compared early and late chronotypes with regards to multiple outcomes. With respect to sleep-wake behaviour, eveningness was associated with higher[90] or equal sleep need,[85] slower increase in sleep pressure during extended wakefulness,[91] later bedtimes and wake-up times (especially on weekends),[92,93] shorter sleep on weekdays and longer duration on weekends achieved by later wake times,[85,93] difficulty in initiating sleep and greater daytime sleepiness,[94] and more frequent naps and poorer sleep quality.[93] The majority of these studies were based on self-assessments using questionnaires to evaluate sleep habits and complaints. The results are somewhat circular as chronotype is assessed by questions asking for sleep-wake behaviour, and basically describe what is used to *define* chronotype. Moreover, many of the reported differences, *e.g.,* poorer sleep and daytime

sleepiness, may as well result from the discrepancy between clock-regulated sleep-wake behaviour and social requirements, as was described previously in the discussion. Some of these studies also reported more irregular sleep-wake schedules[85,90,92,93,95] that again, might be explainable by a greater discrepancy between work and free days in late types leading to a higher variability in bedtime, arising time, and sleep duration due to external constraints. Thus, it is unclear from these studies whether sleep-wake behaviour is intrinsically or artificially more variable. Likewise, this study cannot resolve the question.

Here, the more variable sleep timing in late chronotypes seemed causal for their higher mid-sleep deviations. This finding was further supported by the negative relationship between chronotype and inter-daily stability suggesting that late chronotypes might not be *per se* misaligned, but display a less stable sleep-wake behaviour resulting in higher circadian disruption. To date, it is unclear whether irregular sleep-wake behaviour constitutes a positive or negative factor. On one hand, studies report that both, eveningness and flexibility in sleeping at unusual hours help adaptation to (night) shift work.[96,97] On the other hand it is reported as risk factor for or symptom of (psychiatric) diseases (*i.e.* bipolar disorder[64]). Identifying what causes sleep-wake variability will help clarifying its role in development and prevention of diseases. Here, several potential exogenous and endogenous explanations are proposed.

3.5.3.1 Exogenous causes: Differences in light exposure

Chronotypes might differ in both, degree and regularity of light exposure. Goulet et al.[98] examined daily light exposure in morning-type and evening-type individuals, and found hat morning-types were exposed to more bright light (> 1000 lux) and more light in the morning in relation to external time. Yet, when analysed in relation to circadian phase (as indexed by DLMO), these differences vanished except for individuals with very early or very late

DLMOs, whose profiles of light exposure differed largely. The authors hypothesized that these individuals might have shorter or longer circadian periods, respectively, and a phase-delaying profile of light exposure in morning types and a phase-advancing profile in evening-types might be needed to ensure stable entrainment to the 24h day. Accordingly, a later phase does not necessarily imply weaker entrainment as there is a natural variation in phase of entrainment within species.[99] Yet, since the invention of the Edison bulb in 1879, electrical lighting allows us to work around the clock, and consequently alters circadian synchronisation of the internal clock to the natural light:dark cycle by reducing zeitgeber (time cue) strength.[100] Less daylight in the morning and artificial light during dark hours delays the phase of entrainment in humans, as predicted by circadian entrainment theory[100] and evidenced by laboratory[101,102] as well as field studies[103]. Thus, later sleep on- and offset times might indeed reflect weaker entrainment due to a (possibly self-selected) low-contrast zeitgeber cycle. Accordingly, weaker entrainment might account for higher variability of sleep-wake behaviour in late types, at least in those with delayed phases because of a reduced zeitgeber strength. Similarly, a more irregular light exposure might as well produce varying sleep-wake times, potentially due to differences in lifestyle as Monk and colleagues reported lower lifestyle regularity in evening types.[104]

3.5.3.2 Endogenous causes: Differences in clock properties

Support for endogenous causes comes from a study chronotyping mice via median of activity (MOA).[105] The authors calculated the Qp statistic,[66] an index of oscillatory stationarity almost identical to the inter-daily stability calculated in this study, and found that mice with a later MOA also had less robust activity rhythms. Among potential intrinsic factors, reduced amplitude of the circadian oscillator might account for higher variability in behaviour via greater susceptibility to phase-shifting stimuli, such as light signals.

In 1978, Reinberg and colleagues related the amplitude of several circadian rhythms (*e.g.*, oral temperature) to magnitude of phase-shift after night work.[106] They found a negative correlation, *i.e.* the smaller the amplitude, the greater the phase shift, concluding that a small amplitude of certain circadian rhythms might promote the individuals' ability to phase-shift easily. A laboratory study compared individuals previously identified as either behaviourally resistant or sensitive to sleep deprivation by psychomotor vigilance test performance.[106] During a 38h continuous wakefulness protocol, reduced amplitude of diurnal rhythms in blood-gene expression was observed in resistant subjects suggesting that lower susceptibility to sleep deprivation might be promoted by less stable circadian rhythms.

Brown et al. used human dermal fibroblasts to examine chronotype differences assessed with the MEQ.[108] They found that transcriptional period length was weakly associated with behavioural phase concluding that other factors might influence chronotype as well. Amongst four individuals with 'normal' (~24.5h) period length but opposing chronotypes (two morning and two evening types), amplitude of Rev-erbα transcription rhythm was lower in the morning types. Mathematical modelling showed that reducing general amplitude of the circadian oscillator and decreasing sensitivity of light-signalling pathways, respectively, resulted in phase advance. They further conducted a phase-shifting experiment using a chemical agent and observed that as expected lower amplitudes resulted in larger phase shift magnitudes as shown by phase response curves. Their results suggest that a weaker oscillator is more susceptible to phase-shifting stimuli, possibly explaining a higher variability in sleep timing; yet, they also found that a weaker oscillator (by modelling and by observation in (only) Rev-erbα transcription) was associated with an earlier chronotype contrasting the more variable sleep-wake behaviour that was found here in late types.

Phillips et al. probed the mechanisms of chronotype using a physiologically based mathematical model of the sleep-wake regulatory brain

network.[109] They defined chronotype by preferred sleep-wake schedule, and examined how sleep-wake patterns could be affected by changing model parameters. In agreement with Brown and colleagues,[108] they found that reducing circadian drive (lower general amplitude) thereby increasing homeostatic force resulted in an earlier phase of the sleep-wake behaviour. Furthermore, they demonstrated that two subgroups observed in a previous empirical study[98] could be explained by differing mechanisms: subjects whose dim light melatonin onset (DLMO) did not track their chronotype could be described by differences in homeostatic kinetics (*i.e.* time constant of clearance and accumulation of forebrain adenosine) with later types having slower kinetics, whereas subjects whose DLMO tracked their chronotype could be explained by differences in circadian period τ with later types having longer τ.

Overall, mathematical modelling shows that reducing circadian amplitude thereby mimicking a weaker oscillator results in phase advance. However, in none of these models did a weaker oscillator result in greater cycle-to-cycle variation of sleep-wake behaviour, leaving it still open what causes the higher variability observed in late types.

3.5.4 Limitations, outlook, and conclusions

Daily mid-sleep times were extracted from actimetry and no information on use of alarm clocks was available. Therefore, a potential impact by self-imposed early awakening on results cannot be excluded. Yet, for participants who additionally filled out sleep logs indicating use of alarm clocks, no differences between chronotypes were found regarding frequency of use (data not shown). Future studies are warranted to reveal predictive strength of mid-sleep deviations for health and safety outcomes, and to elucidate the role of sleep-wake variability for development and prevention of diseases.

Mid-sleep deviations offer a simple and promising measure for circadian disruption of sleep-wake behaviour. In view of increasing rates of

people in shift work, it might help to systematically investigate causes and consequences of disrupted rhythms on an individual basis. The study emphasises the importance of chronotype for examining circadian disruption, as the interaction of both, internal and external time will determine who is affected when and to what extent.

3.6 Appendix

3.6.1 Exclusion process

Table A3.1. Number of excluded participants according to criteria and subsamples.

Subsample according to study site and schedule	Initial sample size	Exclusion criteria					Final sample size
		Drop-out	< 2 shifts of each type (M,E,N)	Changes in schedule (> 3 days)	Missing data (> 3 days)	Technical defect	
S1	28	-	-	1	4	-	23
S2	12	-	-	1	2	-	9
S2: 555	11	-	1	1	1	-	8
S3: 222	25	6	1	1	3	1	13
total	76	6	2	4	10	1	53

3.6.2 Descriptive comparison of original and imputed dataset

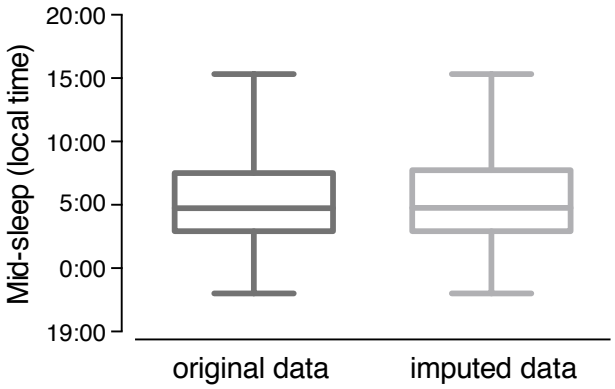

Figure A3.1. Boxplots with median and inter-quartile range are shown before ('original data') and after ('imputed data') imputation of missing values. For details on imputation process, see Section 3.3.4.

3.6.3 Sample description

Table A3.2. Descriptive information of total sample and according to chronotype categories (early, intermediate, late). Chronotype categories were chosen according to inter-quartile range of the sample. MSF^{E}_{sc} = mid-sleep on free days after evening shift, corrected for over-sleep. sd = standard deviation. BMI = body mass index. No significant differences are observed between early, intermediate, and late types regarding age, body mass index, and sleep duration (one-way anovas, $P > 0.05$). Groups differed with respect to chronotype ($F = 111.02$, $P < 0.001$), gender and number of participants not having children (χ^2, $P < 0.05$).

	Total sample (n = 53)	Early (n = 13)	Intermediate (n = 27)	Late (n = 13)
Chronotype (MSF^{E}_{sc})	4:56 ± 91min (1:31 – 8:59)	3:16 ± 37min (1:31 – 3:53)	4:43 ± 32min (3:54 – 5:34)	7:04 ± 53min (5:42 – 8:59)
Age (years, mean ± sd, range)	35 ± 9.61 (19 – 55)	35.31 ± 8.71 (21 – 47)	35.81 ± 10.35 (19 – 55)	33 ± 9.31 (19 – 48)
Gender (% female)	54.72	53.85	62.96	38.46
BMI (mean ± sd, range)	26.19 ± 4.90 (19 – 47)	28.79 ± 7.13 (21 – 47)	25.02 ± 3.60 (19 – 35)	26.03 ± 3.85 (20 – 34)
Children (% without)	60.38	46.15	74.07	58.35
Total sleep duration (hours, mean ± sd, range)	7.14 ± 0.63 (5.49 – 8.61)	6.94 ± 0.73 (5.40 – 8.13)	7.09 ± 0.64 (5.49 – 8.61)	7.29 ± 0.66 (6.23 – 8.42)

3.6.4 Full description of regression models

In this section, full regression models are described including standardised (β) and unstandardised (b) estimates for all parameters entered into the model. They are listed as referenced in the main text. All models included the covariates age, body mass index, gender, number of children (living in the same household) and schedule. The latter three were entered as categorical predictors, coded as follows: gender 0 = female, 1 = male; number of children 0 = no children, 1 = one or more children; schedule 1 = S3: 222 (fastest rotation), 2 = S1, 3 = S2, 4 = S2: 555. The adjusted R^2 value accounts for the number of predictors in the model such that it only increases if a predictor improves (the unadjusted) R^2 more than would be expected by chance. It is therefore considered the more appropriate effect size. P-values were tested against Bonferroni-corrected α – level of 0.006 due to multiple comparisons.

Table A3.3. Regression model for mid-sleep deviation on night shifts. Please note that four participants (MSF^{E}_{sc} = 3:07 / 4:42 / 7:13 / 8:59) displayed outlier values (more than three inter-quartile ranges away from the sample mean) and were therefore excluded from regression analyses. n = sample size. MSF^{E}_{sc} = mid-sleep on free days after evening shift, corrected for over-sleep. BMI = body mass index. se = standard error.

F (df)	P	R^2	Adjusted R^2	n
8.99 (8,40)	< 0.001	0.64	0.57	49

Variable	b	se	t	P > | t |	β
Chronotype (MSF^{E}_{sc})	-0.76	0.10	-7.38	0.000	-0.77
Age	0.02	0.02	1.41	0.168	0.17
Gender	0.64	0.39	1.62	0.113	0.23
BMI	0.01	0.03	0.24	0.813	0.03
Children	-0.61	0.35	-1.73	0.091	-0.22
Schedule					
S1	0.07	0.43	0.15	0.882	0.02
S2	1.18	0.57	2.06	0.046	0.32
S2: 555	0.81	0.49	1.64	0.108	0.22
Constant	8.77	1.09	8.02	0.000	

Table A3.4. Regression model for mid-sleep deviation on free days after night shifts. n = sample size. MSF^{E}_{sc} = mid-sleep on free days after evening shift, corrected for over-sleep. BMI = body mass index. se = standard error.

F (df)	P	R^2	Adjusted R^2	n
4.93 (8,44)	< 0.001	0.47	0.38	53

Variable	b	se	t	P > | t |	β
Chronotype (MSF^{E}_{sc})	-0.55	0.13	-4.32	0.000	-0.53
Age	0.04	0.02	1.80	0.079	0.25
Gender	0.56	0.51	1.10	0.279	0.18
BMI	-0.001	0.04	-0.03	0.977	-0.003
Children	-0.82	0.47	-1.75	0.087	-0.26
Schedule					
S1	-0.46	0.56	-0.82	0.415	-0.15
S2	0.87	0.73	1.19	0.241	0.21
S2: 555	0.74	0.66	1.13	0.265	0.17
Constant	6.84	1.41	4.87	0.000	

Table A3.5. Regression model for mid-sleep deviation on morning shifts. Please note that two participants (MSF^{E}_{sc} = 8:20 / 8:59) displayed outlier values (more than three inter-quartile ranges away from the sample mean) and were therefore excluded from regression analyses. n = sample size. MSF^{E}_{sc} = mid-sleep on free days after evening shift, corrected for over-sleep. BMI = body mass index. se = standard error.

F (df)	P	R^2	Adjusted R^2	n
34.06 (8,42)	< 0.001	0.86	0.84	51

Variable	b	se	t	P > | t |	β
Chronotype (MSF^{E}_{sc})	0.95	0.06	15.81	0.000	0.96
Age	-0.003	0.01	-0.32	0.754	-0.02
Gender	-0.18	0.22	-0.80	0.426	-0.07
BMI	0.01	0.02	0.35	0.732	0.02
Children	-0.01	0.20	-0.06	0.949	-0.004
Schedule					
S1	-0.40	0.24	-1.66	0.103	-0.15
S2	0.20	0.31	0.65	0.517	0.06
S2: 555	-0.52	0.29	-1.82	0.076	-0.14
Constant	-0.64	0.64	-1.00	0.323	

Table A3.6. Regression model for mid-sleep deviation on free days after morning shifts. Please note that the individual study period of five participants comprised no free days after morning shifts reducing the sample size to n = 48. n = sample size. MSF^{E}_{sc} = mid-sleep on free days after evening shift, corrected for over-sleep. BMI = body mass index. se = standard error.

F (df)	P	R^2	Adjusted R^2	n
6.38 (8,39)	< 0.001	0.57	0.48	48

Variable	b	se	t	P > | t |	β
Chronotype (MSF^{E}_{sc})	0.62	0.11	5.60	0.000	0.66
Age	-0.004	0.02	-0.20	0.843	-0.03
Gender	0.67	0.44	1.53	0.135	0.23
BMI	0.03	0.03	0.94	0.352	0.11
Children	-0.50	0.44	-1.15	0.258	-0.17
Schedule					
S1	0.42	0.52	0.81	0.422	0.15
S2	1.39	0.67	2.08	0.044	0.36
S2: 555	0.82	0.60	1.36	0.181	.021
Constant	-0.96	1.29	-0.74	0.465	

Table A3.7. Regression model for overall mid-sleep deviation (MSD_{mean}). Please note that two participants (MSF^{E}_{sc} = 4:42 / 8:20) displayed outlier values (more than three inter-quartile ranges away from the sample mean) and were therefore excluded from regression analyses. n = sample size. MSF^{E}_{sc} = mid-sleep on free days after evening shift, corrected for over-sleep. BMI = body mass index. se = standard error.

F (df)	P	R^2	Adjusted R^2	n
4.86 (8,42)	< 0.003	0.48	0.38	51

Variable	b	se	t	P > | t |	β
Chronotype (MSF^{E}_{sc})	0.17	0.04	3.73	0.001	0.46
Age	0.01	0.001	-0.20	0.180	0.20
Gender	0.33	0.17	1.92	0.062	0.31
BMI	0.01	0.01	1.04	0.303	0.13
Children	-0.21	0.16	-1.31	0.197	-0.20
Schedule					
S1	-0.39	0.20	-2.00	0.051	-0.37
S2	0.27	0.25	1.06	0.297	0.19
S2: 555	-0.09	0.23	-0.39	0.700	-0.06
Constant	1.98	0.50	3.97	0.000	

Table A3.8. Regression model for 'window of tolerance' for MSD_i. The window included all values within a range of ± 2h and the percentage of values outside this window was calculated. Please note that one participant (MSF^E_{sc} = 8:20) displayed an outlier value (more than three inter-quartile ranges away from the sample mean) and was therefore excluded from regression analyses. n = sample size. MSF^E_{sc} = mid-sleep on free days after evening shift, corrected for over-sleep. BMI = body mass index. se = standard error.

F (df)	P	R^2	Adjusted R^2	n
7.86 (8,43)	< 0.001	0.59	0.52	52

Variable	b	se	t	P > | t |	β
Chronotype (MSF^E_{sc})	0.07	0.01	7.00	0.000	0.75
Age	0.003	0.002	1.59	0.120	0.20
Gender	0.05	0.04	1.37	0.178	0.20
BMI	0.0006	0.003	0.23	0.819	0.02
Children	-0.06	0.03	-1.92	0.062	-0.25
Schedule					
S1	-0.07	0.04	-1.75	0.087	-0.28
S2	-0.01	0.05	-0.26	0.796	-0.04
S2: 555	-0.04	0.04	-0.83	0.409	-0.11
Constant	0.21	0.10	1.98	0.054	

Table A3.9. Regression model for variability of mid-sleep deviations (MSD_{var}). Please note that two participants (MSF^{E}_{sc} = 6:30 / 7:13) displayed outlier values (more than three inter-quartile ranges away from the sample mean) and were therefore excluded from regression analyses. n = sample size. MSF^{E}_{sc} = mid-sleep on free days after evening shift, corrected for over-sleep. BMI = body mass index. se = standard error.

F (df)	P	R^2	Adjusted R^2	n
7.55 (8,42)	< 0.001	0.59	0.51	51

Variable	b	se	t	P > | t |	β
Chronotype (MSF^{E}_{sc})	0.16	0.02	7.60	0.000	0.85
Age	-0.001	0.004	-0.34	0.737	-0.04
Gender	-0.13	0.08	-1.66	0.105	-0.25
BMI	-0.0004	0.006	-0.07	0.941	-0.008
Children	-0.003	0.07	-0.04	0.971	-0.004
Schedule					
S1	-0.31	0.09	-3.43	0.001	-0.57
S2	-0.29	0.11	-2.55	0.015	-0.41
S2: 555	-0.29	0.10	-2.87	0.006	-0.40
Constant	1.01	0.22	4.70	0.000	

Table A3.10. Regression model for variability of mid-sleep deviations (MSD_{var}) including two additional predictors regarding deviations and variability of sleep duration (SDD_{mean} and SDD_{var}). Please note that two participants (MSF^{E}_{sc} = 6:30 / 7:13) displayed outlier values (more than three inter-quartile ranges away from the sample mean) and were therefore excluded from regression analyses. n = sample size. MSF^{E}_{sc} = mid-sleep on free days after evening shift, corrected for over-sleep. SDD_{mean} = overall sleep duration deviation. SDD_{var} = variability of sleep duration deviation. BMI = body mass index. se = standard error.

F (df)	P	R^2	Adjusted R^2	n
5.99 (10,40)	< 0.001	0.60	0.50	51

Variable	b	se	t	P > | t |	β
Chronotype (MSF^{E}_{sc})	0.15	0.02	7.08	0.000	0.83
SDD_{mean}	0.09	0.10	0.96	0.345	0.21
SDD_{var}	0.07	0.08	0.91	0.366	0.20
Age	-0.001	0.004	-0.37	0.712	-0.05
Gender	-0.12	0.08	-1.46	0.153	-0.22
BMI	0.0003	0.006	0.05	0.960	0.005
Children	-0.009	0.07	-0.12	0.908	-0.02
Schedule					
S1	-0.29	0.09	-3.06	0.004	-0.53
S2	-0.25	0.12	-2.04	0.048	-0.36
S2: 555	-0.25	0.11	-2.24	0.031	-0.34
Constant	0.46	0.61	0.75	0.459	

Table A3.11. Regression model for overall mid-sleep deviations (MSD_{mean}) including two additional predictors regarding deviations and variability of sleep duration (SDD_{mean} and SDD_{var}). Please note that two participants (MSF^{E}_{sc} = 4:42 / 8:20) displayed outlier values (more than three inter-quartile ranges away from the sample mean) and were therefore excluded from regression analyses. n = sample size. MSF^{E}_{sc} = mid-sleep on free days after evening shift, corrected for over-sleep. SDD_{mean} = overall sleep duration deviation. SDD_{var} = variability of sleep duration deviation. BMI = body mass index. se = standard error.

F (df)	P	R^2	Adjusted R^2	n
4.05 (10,40)	< 0.007	0.50	0.39	51

Variable	b	se	t	P > | t |	β
Chronotype (MSF^{E}_{sc})	0.16	0.05	3.54	0.001	0.45
SDD_{mean}	0.19	0.21	0.88	0.384	0.22
SDD_{var}	0.04	0.18	0.25	0.806	0.06
Age	0.01	0.008	1,55	0.129	0.23
Gender	0.33	0.18	1.86	0.070	0.31
BMI	0.02	0.01	1.14	0.261	0.14
Children	-0.22	0.16	-1.36	0.182	-0.21
Schedule					
S1	-0.31	0.21	-1.54	0.133	-0.30
S2	0.39	0.27	1.41	0.166	0.28
S2: 555	0.04	0.25	0.14	0.886	0.02
Constant	1.16	1.32	0.88	0.387	

4 Project Three

Challenging current guidelines for night and shift work

4.1 Brief summary

Current European guidelines on night and shift work give several recommendations for the design of shift schedules aiming at the reduction of adverse effects on health, safety, and social life. Accordingly, slow rotations and more than three consecutive night shifts should be avoided. Yet, analyses considering individual characteristics, such as chronotype, are missing but will potentially add to existing guidelines. Here, the effects of single shift sequences were examined taking into account individual chronotype. Ninety-seven employees (31% female, age 36 ± 10 years, body mass index 26 ± 5) working in seven different rotating shift schedules filled out sleep logs daily over the course of at least four weeks. Sleep duration on work and work-free days, and a new measure quantifying circadian disruption of the sleep-wake cycle ('mid-sleep deviations') were calculated from sleep logs. Mixed-effects regression models and rank correlations were conducted to compare different numbers of consecutive (morning, evening, night) shifts. Results show an interaction effect between type of shift, chronotype, and number of successive shifts: with more night shifts (*i.e.* 2 *vs.* 4 night shifts), both, mid-sleep deviations and the difference between sleep duration on work and work-free days increased for early and decreased for late chronotypes, indicating an improved circadian alignment in late types when exposed to more than two night shifts in a row. The findings suggest that the number of consecutive shifts beneficial for an individual depends strongly on chronotype. The results of this study are intended to serve a science-based evaluation and

optimisation of current shift work guidelines, particularly with regard to individual internal time.

4.2 Introduction

Work schedules can have dramatic effects on sleep timing and sleep duration, especially in rotating shift schedules where employees have not only one, but several working times to cope with. In order to reduce adverse effects on health, social life, and safety, current European guidelines on night and shift work compile several recommendations for the design of shift schedules.[1–4] These guidelines advise, for example, quickly and forward rotating shift schedules, avoiding an early start of the morning shift, and limiting the number of successive night shifts to three or less. In general, recommendations root in evidence from field and laboratory studies, such as start of morning shifts preferably after 6 a.m.,[5,6] and forward direction of rotating schedules, as circadian rhythms were shown to adjust ~50% faster to phase delays than phase advances[7] (although recent studies suggests that counter-clockwise rotations might not be as harmful as early research indicated[8]). Yet, taking into account individual characteristics influencing adaptation to (night) shift work might optimise guidelines. Among these, individual chronotype appears an important factor for 'shift work tolerance' (*i.e.* absence of adverse effects) as it was shown to modulate cognitive performance, sleep duration and social jetlag (a measure for circadian misalignment) in rotational shift workers.[9,10] Late chronotypes perform better, sleep longer and experience less social jetlag on night shifts compared with early chronotypes. Furthermore, previous analyses with a newly developed measure of mistimed sleep ('mid-sleep deviations', see Section 3 Project Two) revealed a higher variability of sleep-wake behaviour in late than early chronotypes potentially enhancing their adaptation to not only late working times but (rotational) shift work in general. A higher variability in sleep timing could also play a role when evaluating

effects of rotation speed as a faster adaptation to varying working times might mitigate negative outcomes, such as fatigue and sleep problems.[11,12] Yet, determination of rotation speed is especially challenging in irregular shift schedules where each type of shift has its own periodicity (*e.g.,* 6 morning, 2 evening, 3 night shifts). Detailed analyses of shift sequences (*i.e.* blocks of consecutive workdays) within a schedule would allow for evaluating the number of consecutive shifts beneficial for an individual, and possibly add to current ergonomic guidelines. Such analyses are missing, particularly with regard to individual internal time, *i.e.*, chronotype.

Here, single shift sequences were examined with regards to individual chronotype and sleep-wake behaviour comparing different numbers of consecutive shifts. Based on previous studies, it was hypothesised that increasing the number of consecutive night shifts would strengthen adverse effects in early types but foster adaptation in late types; in turn, a higher number of morning shifts was expected to impact negatively on sleep-wake behaviour in late types while having positive influence in early ones.

4.3 Methods

4.3.1 Study design and shift schedules

Sleep log data were merged from three different studies including seven different schedules in total. First study with schedule 'S1' took place in 2008 (Cham, Germany), second study was conducted in 2009 involving schedule 'S2' (Berlin, Germany), and the third study was carried out in 2012 (Bochum, Germany) accompanying the implementation of a new *chronotype-adapted* shift system ('*CTA* schedule'). In the original system, all employees worked the same fast-forward rotating schedule ('S3: 222'), while in the new system shift workers were assigned to one of four groups according to their individual chronotypes with each group working a different sequence of shifts (resulting in four schedules 'S3: E1/E2/L1/L2'). The earliest quarter of the chronotype

distribution at study site 3 was assigned to shift group ‘Early 1’ (S3: E1), the middle 50% to groups ‘Early 2’ (S3: E2) and ‘Late 1’ (S3: L1), and the latest 25% worked in group ‘Late 2’ (S3: L2) (for more details, please see[13]). While E2 and L1 experienced only modest alterations to their schedule, E1 and L2 comprising the most extreme chronotypes also had the largest changes: night shifts in E1 and morning shifts in L2 were completely abolished.

Overall, analyses included three subsamples (Cham: $n_1 = 34$, Berlin: $n_2 = 23$, Bochum: $n_3 = 40$) adding up to 97 shift workers in total, with repeated measures at study site 3 before, after and during implementation of the new CTA schedule ($n_{3:E1} = 14$, $n_{3:E2} = 10$, $n_{3:L1} = 6$, $n_{3:L2} = 10$). All schedules were forwards-rotating, involved 8-h shifts, and had standard European transition times (morning shift: 6 a.m. – 2 p.m., evening /afternoon shift: 2 p.m. – 10 p.m., night shift: 10 p.m. – 6 a.m.). Figure 4.1. illustrates the different schedules analysed in Project Three.

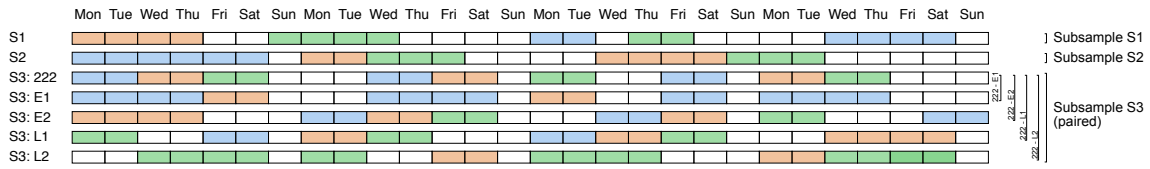

Figure 4.1. Rotational shift schedules. Participants’ data were merged from three independent subsamples (S1, S2, S3) in seven different forwards-rotating schedules. Please note that shift workers in subsample S3 experienced a change of shift system: the original ‘S3: 222’ schedule was replaced with a chronotype-adapted system where each group worked a different schedule according to employees’ chronotype (‘S3: E1/E2/L1/L2’). Thus, shift workers’ data from study site S3 were paired with each participant working schedule ‘S3: 222’ followed by one of the four CTA schedules (E1, E2, L1, or L2).

4.3.2 Chronotype calculation

Following the calculations of the Munich Chronotype Questionnaire for Shift-Workers (MCTQ^{Shift} [14]), participants’ individual chronotype was determined from sleep logs using mid-sleep on free days after evening shifts (corrected for potential over-sleep) as a proxy (MSF^{E}_{sc}). In case there were less than two

free days after evening shifts reported in the sleep log, a transformation algorithm proposed by Juda et al.[14] was applied to estimate chronotype from free days after night shifts.

4.3.3 Sleep logs

In order to increase statistical power for mixed-effects regression analyses (see 4.3.5), sleep log data were used given that sample size was almost doubled compared to actimetry data. Shift workers filled out sleep logs daily for a period of 4 weeks (S1, S2) and 12 weeks (S3, three time points à 4 weeks once before, once directly after, and once at the end of the new CTA schedule, see 4.3.1), respectively. They reported time of preparing to fall asleep, minutes required to fall asleep (sleep latency), time of awakening, time of getting up, and use of alarm clocks, as well as whether the respective day at wake-up was a work-free day or a workday (morning, evening, night shift). Daily sleep durations and mid-points of sleep were used to calculate (i) difference in sleep duration between workdays and work-free days ('differential sleep duration'), and (ii) 'mid-sleep deviations', a newly developed method to determine circadian disruption of the sleep-wake cycle (for details, see previous Section 3 Project Two).

Differential sleep duration was assessed because most people tend to accumulate a certain sleep debt during the workweek that they eventually compensate for on their work-free days by sleeping in.[15–17] The resulting discrepancy between sleep duration on workdays and sleep duration on work-free days thus reflects socially induced sleep deprivation. Comparing absolute sleep duration on workdays without considering sleep on days off will potentially miss effects in individuals with different levels of sleep need. The difference of sleep duration on workdays and days off ('differential sleep duration' or 'Δ sleep duration') was calculated as the absolute difference between averaged sleep duration for successive workdays and sleep duration on first subsequent day off according to the following formula:

$$\Delta \text{ Sleep duration} = \left| \frac{\sum_{i=1}^{n} SD_i^w}{n^w} - SD_j^f \right| \quad \text{(E4.1)}$$

where

SD^w_i = sleep duration on workday i (*i.e.* morning, evening or night shift)
n^w = number of successive workdays (*i.e.* sequence of shifts framed by days off)
SD^f_j = sleep duration on first free day j after n workdays

Please note, that differential sleep duration was only calculated if participants woke up without using an alarm clock on their days off as indicated in the sleep logs.

Mid-sleep deviations were calculated via the length of a two-dimensional vector using Pythagoras' theorem where variable x_i is the difference between mid-sleep on day i and individual chronotype (MSF^E_{sc}), and variable y_i is the distance between mid-sleep on day i and previous mid-sleep (for more details, see previous Section 3 Project Two):

$$\Delta \text{ Chronotype: } x_i = MSF^E_{sc} - MS_i \quad \text{(E4.2)}$$

$$\Delta \text{ Day before: } y_i = MS_{i-1} - MS_i \quad \text{(E4.3)}$$

$$\text{Mid-sleep deviation: } \left| \overrightarrow{MSD_i} \right| = \left| \begin{pmatrix} x_i \\ y_i \end{pmatrix} \right| = \sqrt{x_i^2 + y_i^2} \quad \text{(E4.4)}$$

where

MSF^E_{sc} = chronotype (mid-sleep on free days after evening shifts, corrected for over-sleep)
MS_i = mid-sleep on day i
MSD_i = mid-sleep deviation on day i

4.3.4 Shift sequences

In order to account for the fact that shift schedules may be neither fast nor slow rotating but often involve sequences of different numbers of consecutive shifts (*i.e.* schedule S2 comprises six morning shifts [days off], and two evening shifts followed by three night shifts), schedules were divided into blocks of consecutive workdays considered as 'shift sequences'. Thus, a shift sequence (i) is framed by days off, (ii) can comprise different types of shifts, and (iii) can be of varying length. Splitting the seven schedules into blocks of successive workdays results in 14 different shift sequences that were further clustered according to the number of consecutive shifts before the first free day (*i.e.* all sequences that end on two night shifts before days off were grouped together). Reducing the number of sequences by clustering was applied to ensure adequate regression modelling increasing statistical power and facilitating interpretation. Finally, eight units of shift sequences resulted: sequences ending on two (2M), four (4M), or six morning shifts (6M); two (2E) or four evening shifts (4E); and two (2N), three (3N), or four night shifts (4N). Figure 4.2. illustrates the approach.

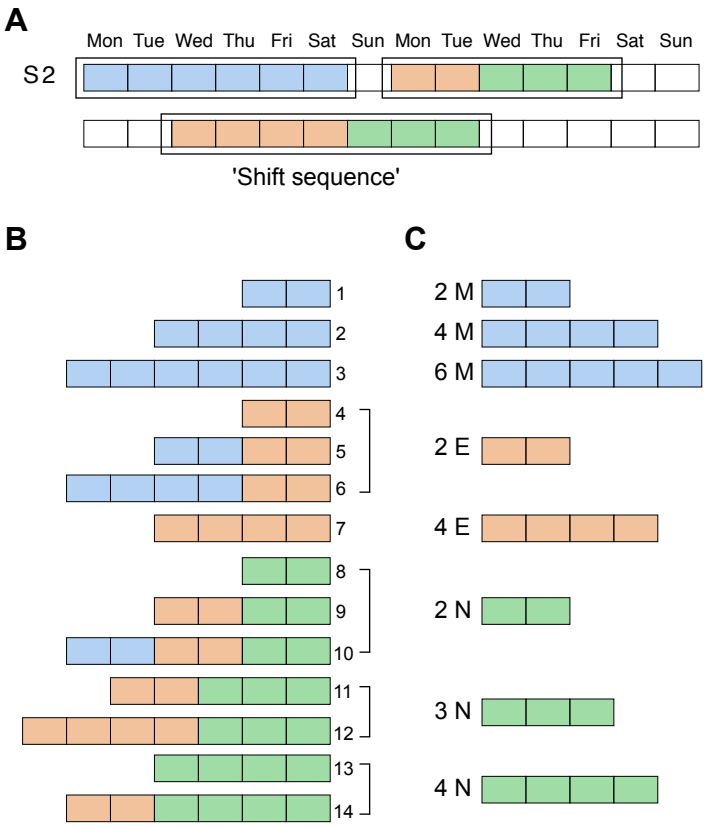

Figure 4.2. Shift sequences. **(A)** Each schedule was divided into 'shift sequences', blocks of consecutive workdays framed by days off, *i.e.* schedule S2 consisted of three such sequences. **(B)** Fourteen different sequences emerged from the seven schedules and were grouped according to the type of shift before the next free day (morning = blue boxes, evening = orange boxes, night shift = green boxes). **(C)** Because fourteen sequences were too many to be entered into the regression model, they were further clustered by the number of consecutive shifts, *i.e.* sequences 4, 5, and 6 were combined into the unit of two successive evening shifts ('2 E').

4.3.5 Data set structure and statistical analyses

Longitudinal shift work data from field studies pose several challenges to statistical analyses: missing values, nested data structure (subjects within shift groups within shift schedules), and complex variance-covariance patterns. Mixed models provide a flexible approach to explicitly model those situations. Here, both, fixed and random effects were specified to analyse shift sequences with regards to sleep-wake behaviour. First, indicator variables were created to mark start, end and length of each sequence (*i.e.* 2 night

shifts). Next, sequences of the same type of shift but different numbers of consecutive shifts (*i.e.* 2, 3, and 4 night shifts) were combined into one single factor (*i.e.* night shift factor). Thus, the reference group comprised all entries not being part of the respective sequences (see Table 4.1). Separate mixed models were computed for each type of shift (morning, evening, night shift). Sequence factors, chronotype as well as their interaction term were entered as fixed effects with shift schedule, age, gender, body mass index, overall sleep duration, time of sunrise and photoperiod as covariates. Random effects were specified for subjects and sequences to allow both, random intercepts and random slopes. No assumptions were made about the variance-covariance matrix, therefore chosen to be 'unstructured' in the model. In case fixed effects or covariates were non-significant, they were removed from the model and analysis was re-conducted. For analyses of differential sleep duration, Spearman's rho rank correlations were conducted to account for non-normality as confirmed by Shapiro-Wilk tests. Data processing and analyses were done in STATA (Stata/SE 12.0), and plots were drawn in Prism (GraphPad Software 6.0) and idraw (Indeeo Inc., 2.4).

Table 4.1. Exemplified data structure for mixed model analyses. First three columns contain information on subject, shift schedule and type of shift (morning (M), evening (E), night (N) shift) with multiple measures per subject. For each shift sequence, a column was created representing an indicator variable for start and end of respective sequence (*i.e.* night shift sequences 8, 9, 11, and 13, see Fig. 4.2). Finally, in the last column, all (*i.e.* night) shift-related sequences were grouped into one single sequence factor (*i.e.* 'night shift factor'), with cipher '1' = two consecutive night shifts, '2' = three consecutive night shifts, '3' = four consecutive night shifts, and '0' = reference observations. The single sequence factor was then entered into the regression model.

Subject	Schedule	Type of shift	Seq. 8	Seq. 9	Seq. 11	Seq. 13	Seq.factor 'Night shift'
1	S1	-	0	0	0	0	0
1	S1	N	1	0	0	0	1
1	S1	N	1	0	0	0	1
1	S1	-	0	0	0	0	0
…	…	…	…	…	…	…	…
2	S3: 222	-	0	0	0	0	0
2	S3: 222	E	0	1	0	0	0
2	S3: 222	E	0	1	0	0	0
2	S3: 222	N	0	1	0	0	1
2	S3: 222	N	0	1	0	0	1
2	S3: 222	-	0	0	0	0	0
…	…	…	…	…	…	…	…
3	S2	-	0	0	0	0	0
3	S2	E	0	0	1	0	0
3	S2	E	0	0	1	0	0
3	S2	N	0	0	1	0	2
3	S2	N	0	0	1	0	2
3	S2	N	0	0	1	0	2
3	S2	-	0	0	0	0	0
…	…	…	…	…	…	…	…
4	S3: L2	-	0	0	0	0	0
4	S3: L2	N	0	0	0	1	3
4	S3: L2	N	0	0	0	1	3
4	S3: L2	N	0	0	0	1	3
4	S3: L2	N	0	0	0	1	3
4	S3: L2	-	0	0	0	0	0
…	…	…	…	…	…	…	…

4.4 Results

Analyses of evening shift sequences revealed no significant effects regarding differential sleep duration and mid-sleep deviations, respectively. Except for section 4.4.1, they were therefore excluded from the main Results part, but can be found in the Appendix of this project (see 4.6). Table 4.2 shows demographic information of the sample.

Table 4.2. Demographic description of total sample and according to study site subsamples (S1, S2, S3). MSF^{E}_{sc} = mid-sleep on free days after evening shift, corrected for over-sleep. sd = standard deviation. BMI = body mass index. Subsamples differed with respect to age and gender (F/χ^2, $P < 0.05$).

	Total sample (n = 97)	S1 (n = 34)	S2 (n = 23)	S3 (n = 40)
Chronotype (MSF^{E}_{sc})	4:33 ± 73min (2:35 – 8:37)	4:59 ± 78min (2:40 – 8:37)	4:28 ± 67min (2:43 – 6:20)	4:14 ± 65min (2:35 – 7:19)
Age (years, mean ± sd, range)	36.24 ± 10.23 (21 – 57)	29.42 ± 8.12 (21 – 47)	36.96 ± 8.34 (25 – 52)	39.93 ± 10.48 (21 – 57)
Gender (% female)	31.27	61.76	34.78	2.50
BMI (mean ± sd, range)	26.04 ± 4.65 (18 – 47)	24.47 ± 5.04 (20 – 41)	25.87 ± 3.16 (20 – 32)	27.08 ± 4.98 (18 – 47)
Children (% without)	51.54	52.94	47.83	52.50
Total sleep duration (hours, mean ± sd, range)	6.95 ± 0.73 (5.11 – 8.61)	6.94 ± 0.73 (5.40 – 8.13)	6.73 ± 0.68 (5.11 – 8.10)	7.07 ± 0.73 (5.16 – 8.61)

4.4.1 Sequence description

Each shift sequence was plotted as a function of sleep duration and mid-sleep deviations showing a systematic relationship: the longer sleep duration, the lower mid-sleep deviations (Fig. 4.3). Participants slept longest and showed lowest deviations on evening shift sequences (sleep duration = $8h_{2E}$ / 7h $51min_{4E}$, mid-sleep deviations = 1h $7min_{2E}$ / $51min_{4E}$). On morning shift sequences, mid-sleep deviations were decreased but shift workers slept less than on night shift sequences (morning shift sequences: sleep duration = 5h $22min_{2M}$ / 5h $36min_{4M}$ / 5h $14min_{6M}$, mid-sleep deviations = 1h $56min_{2M}$ / 1h $39min_{4M}$ / 1h $43min_{6M}$). Interestingly, only night shift sequences differed according to number of consecutive shifts with higher numbers of night shifts relating to increased sleep durations and lower mid-sleep deviations (sleep duration = 5h $33min_{2N}$ / 6h $03min_{3N}$ / 6h $24min_{4N}$, mid-sleep deviations = 4h $19min_{2N}$ / 4h $11min_{3N}$ / 3h $35min_{4N}$).

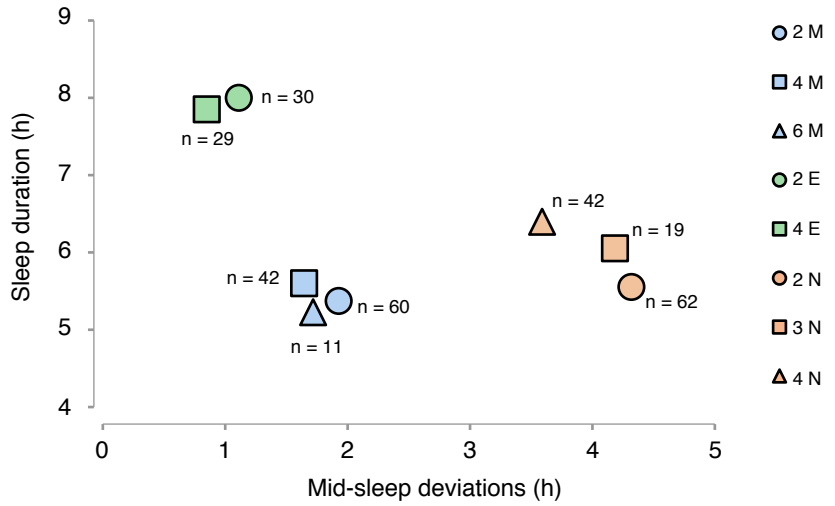

Figure 4.3. Shift sequences according to sleep duration and mid-sleep deviations. Average values across all participants are shown for respective sequences. n = number of participants.

4.4.2 Sleep duration between work and work-free days

Differences in sleep duration between workdays and days off were analysed separately for morning and night shift sequences (for evening shift sequences, see Appendix of this project, 4.6.1). Positive rank correlations were observed for morning shift sequences irrespective of the number of consecutive shifts: the later the chronotype, the larger the difference between sleep duration on morning shifts and subsequent day off (up to 8 hours difference, r = 0.12 – 0.53) (Fig. 4.4). Increasing the number from two to four successive morning shifts did not affect differential sleep duration ($r = 0.53_{2M}$ *vs.* 0.53_{4M}, $P > 0.05$). When working six morning shifts in a row, correlation weakened to non-significance ($r = 0.12$, $P > 0.05$) indicating that later chronotypes might have slept longer on average thus needing less catch-up sleep on their day off (compared with two or four successive morning shifts). Yet, only eleven participants worked the shift sequence of six successive morning shifts. Analyses of night shift sequences revealed negative rank correlations: the earlier the chronotype, the larger the gap between sleep duration on night shifts and first day off irrespective of the number of night shifts worked in a row ($r = -0.24$ – -0.73, $P < 0.05$). The correlation got stronger with a higher number of consecutive night shifts ($r = -0.24_{2N}$ *vs.* -0.73_{4N}, $P < 0.05$). This finding indicates that working more than two successive night shifts worsened the situation of early types but improved the one of late types in terms of sleep duration and deprivation.

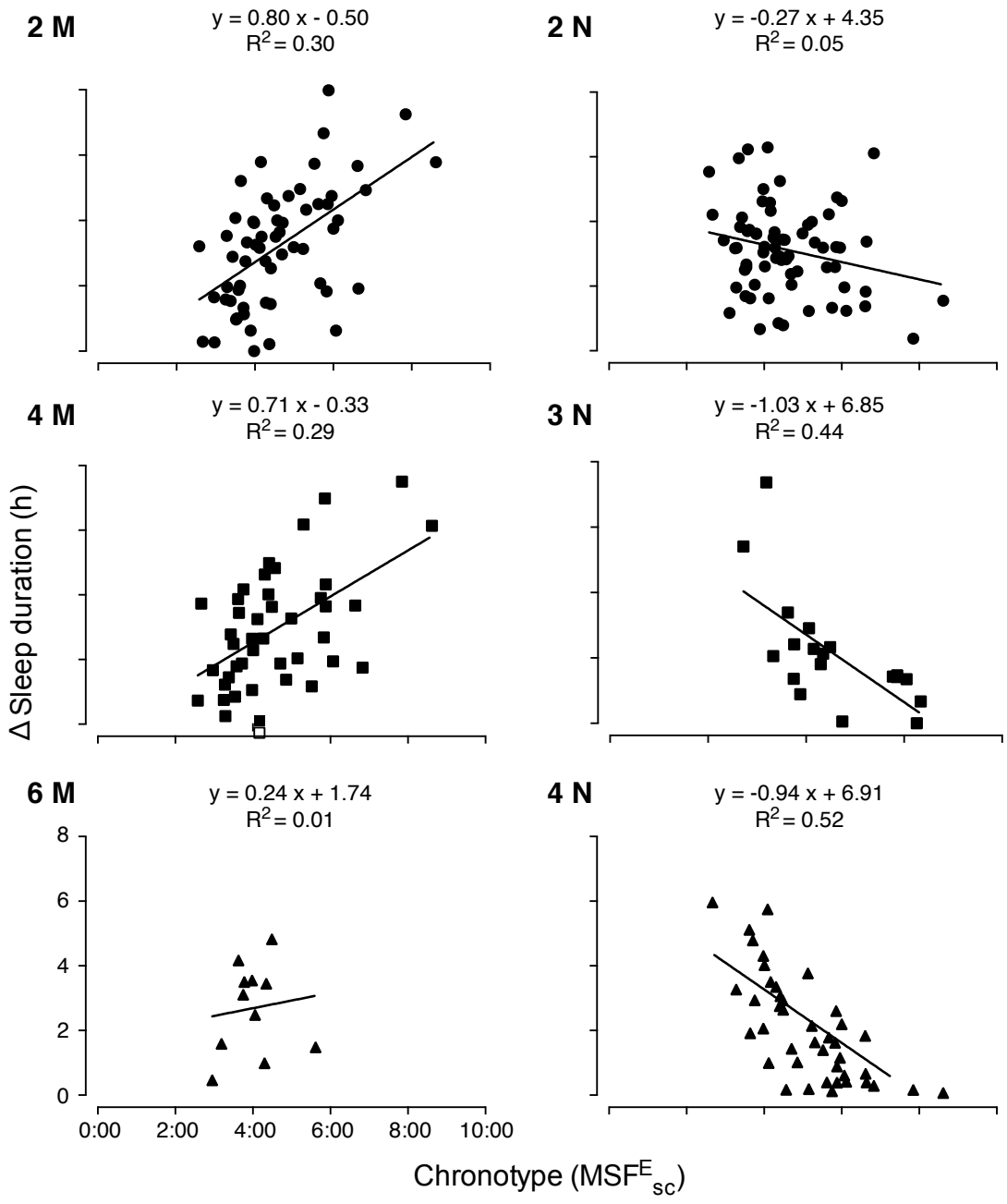

Figure 4.4. Difference in sleep duration on workdays and work-free days ('Δ sleep duration') for morning (2,4,6 M, left panels) and night shift sequences (2,3,4 N, right panels). Equations of trend lines and explained variance (R^2) are shown above each panel.

The lack of change in differential sleep duration from two to four morning shifts as well as the relatively large change from two to four night shifts can result from various patterns of sleep duration on workdays and work-free days. It might be that increasing the number of consecutive morning shifts decreased (or increased) both, sleep duration on workdays and days off leaving differential sleep duration unaffected. Likewise, a stronger relationship between chronotype and differential sleep duration when working a higher number of successive night shifts could stem from a possibly shortened sleep duration on free days while sleep on night shifts might have remained unchanged.

Sleep duration was therefore analysed separately on workdays and work-free days for each morning and night shift sequence. In general, less sleep on workdays was associated with longer sleep durations on days off (Fig. 4.5). With regards to morning shift sequences, sleep duration on both, workdays and work-free days, remained almost constant when increasing the number of consecutive morning shifts from two to four shifts. Yet, the weakened correlation for six successive morning shifts appeared to be based on one participant in this subsample, a chronotype of 5:37 ($MSF^{E}_{sc,}$ German time) who slept only 5 h on first day off despite a very short sleep duration on the six preceding morning shifts of 3.52 h on average (Fig. 4.5, panel '6 M'). Regarding night shift sequences, increasing the number of successive night shifts showed indeed that the later the chronotype, the longer sleep on workdays ($r = 0.61_{3N} / 0.57_{4N}$, $P < 0.05$) and the shorter sleep on work-free days ($r = -0.37_{3N} / -0.38_{4N}$, $P < 0.05$), suggesting that sleep deprivation diminished in late types but increased in early types.

Finally, regression line formulas were equated and solved for night shift sequences '2N' and '4N' revealing that a chronotype of ≥ 3:49 can potentially benefit from an increased number of consecutive night shifts with regards to differential sleep duration.

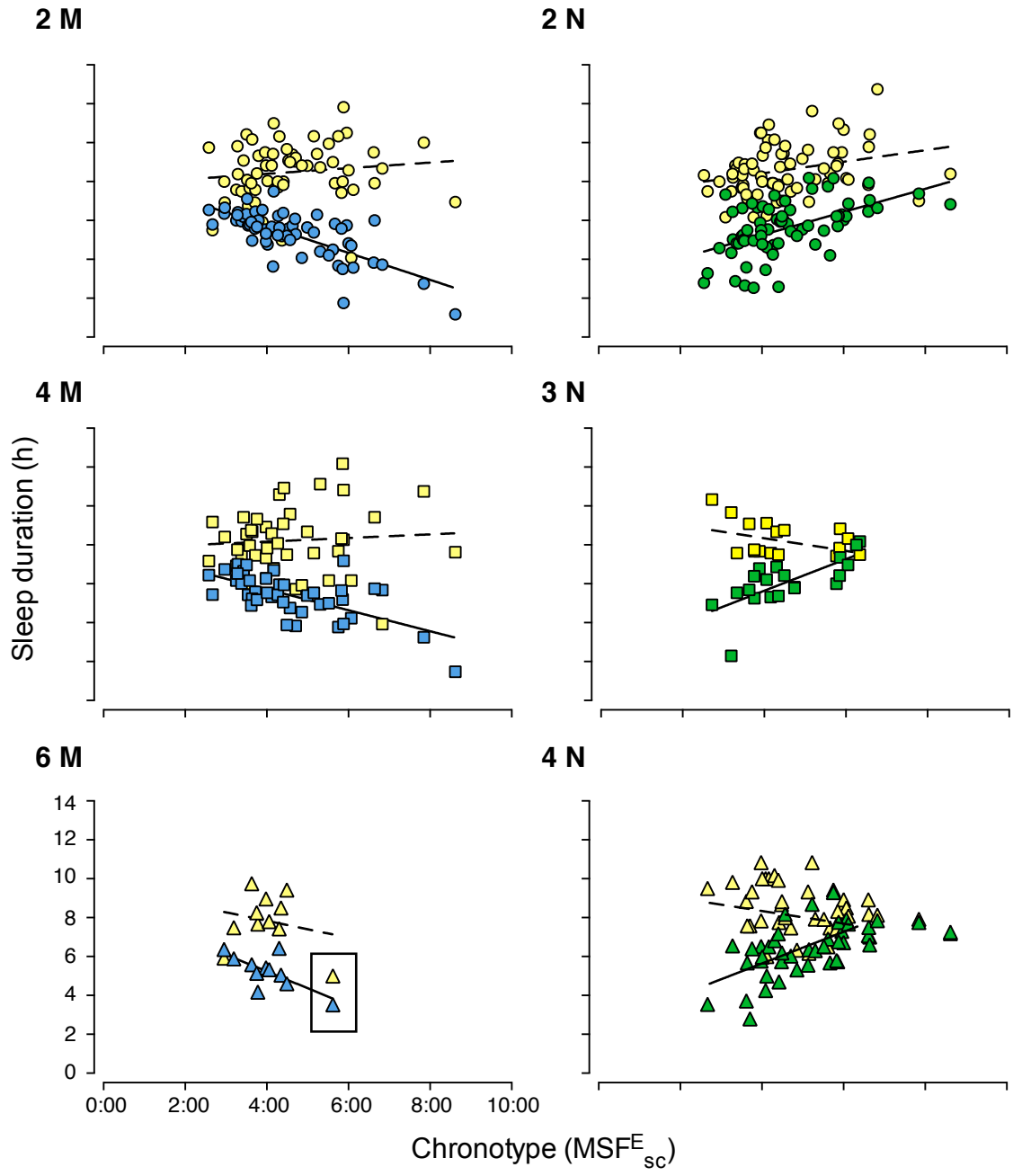

Figure 4.5. Sleep duration on workdays (morning shift = blue symbols, night shift = green symbols) and work-free days (yellow symbols) for morning (2,4,6 M, left panels) and night shift sequences (2,3,4 N, right panels). Straight line = trend on workdays, dotted line = trend on work-free days. The black box in the lower left panel ('6 M') frames one participant (MSF^{E}_{sc} = 5:37) in this subsample potentially biasing the relationship.

4.4.3 Mid-sleep deviations

With regards to circadian disruption of the sleep-wake behaviour measured by the concept of mid-sleep deviations, mixed models were conducted separately for morning and night shift sequences (for evening shift sequences, see Appendix of this project, 4.6.2). Regarding morning shift sequences, chronotype had no significant influence on mid-sleep deviations (unstandardized coefficient, b = 0.06, P > 0.05) (Table 4.3). Mid-sleep deviations decreased when working two and four consecutive morning shifts, respectively, as compared with reference group (*i.e.* no morning shift sequences); yet, the decrease was smaller for four than two successive morning shifts ($b = -3.44_{2M}$ *vs.* -2.34_{4M}, $P < 0.05$). The sequence of six consecutive morning shifts did not reach significance (subsample size: n = 11). Most importantly, significant interaction terms were observed: mid-sleep deviations increased the later the chronotype, but this increase declined as the number of consecutive shifts was raised ($b = 0.77_{2M*chronotype}$ *vs.* $0.55_{4M*chronotype}$, $P < 0.05$).

Table 4.3. Mixed-effects regression model for morning shift sequences (2, 4, 6 consecutive morning shifts (M)). Please note that covariates age, gender, body mass index, overall sleep duration, time of sunrise, and photoperiod had no significant model contribution and were thus removed. The covariate 'shift schedule' lost its significance when re-conducting the analysis but is reported here, as each model was re-conducted only once. Estimate = unstandardised regression coefficient. Se = standard error. CI = confidence interval. S1 / S2 / S3 = shift schedules from different study sites (see 4.3.1).

Variable	Estimate	Se	t	P > | t |	CI (95%)	
					Lower bound	Upper bound
Intercept	3.10	1.93	1.61	.107	-0.67	6.88
Chronotype (MSF^{E}_{sc})	0.06	0.43	0.14	.890	-0.78	0.90
2 M	-3.44	0.69	-4.98	.000	-4.79	-2.08
4 M	-2.34	0.73	-3.21	.001	-3.76	-0.91
6 M	-1.49	2.39	-.632	.534	-6.18	3.20
2 M * chronotype	0.77	0.15	5.29	.000	0.49	1.06
4 M * chronotype	0.55	0.15	3.55	.000	0.24	0.85
6 M * chronotype	0.33	0.59	0.56	.577	-0.83	1.49
S1	-0.35	1.15	-0.31	.760	-2.61	1.91
S2	0					
S3: 222	0.21	1.05	0.20	.838	-1.84	2.27
S3: E1	-1.57	1.05	-1.50	.134	-3.63	0.48
S3: E2	-0.26	1.05	-0.25	.802	-2.32	1.80
S3: L1	0.06	1.06	0.06	.953	-2.01	2.13
S3: L2	1.32	1.05	1.26	.209	-0.74	3.38

Conducting the mixed model for night shift sequences showed similar effects. Again, chronotype had no significant influence (b = 0.07, P > 0.05) (Table 4.4). Mid-sleep deviations increased when working consecutive night shifts as compared with reference group (*i.e.* no night shift sequences), and the increase was larger the higher the number of consecutive shifts ($b = 8.03_{2N}$ *vs.* 8.48_{3N} *vs.* 9.32_{4N}, $P < 0.05$). Additionally, significant interaction effects were observed indicating that mid-sleep deviations decreased the later the chronotype, and even more so when working a higher number of consecutive night shifts ($b = -0.91_{2N*chronotype}$ *vs.* $-0.96_{3N*chronotype}$ *vs.* $-1.16_{4N*chronotype}$, $P < 0.05$).

Table 4.4. Mixed-effects regression model for night shift sequences (2, 3, 4 consecutive night shifts (N)). Please note that none of the covariates age, gender, body mass index, shift schedule, overall sleep duration, time of sunrise, and photoperiod contributed significantly and were therefore removed from the model. Estimate = unstandardised regression coefficient. Se = standard error. CI = confidence interval. S1 / S2 / S3 = shift schedules from different study sites (see 4.3.1).

Variable	Estimate	Se	t	P > | t |	CI (95%)	
					Lower bound	Upper bound
Intercept	1.48	0.48	3.10	.002	0.54	2.41
Chronotype (MSF^{E}_{sc})	0.07	0.10	0.70	.784	0.07	0.47
2 N	8.03	0.69	11.59	.000	6.60	9.47
3 N	8.48	0.60	14.26	.000	7.31	9.65
4 N	9.32	1.20	7.74	.000	6.91	11.72
2 N * chronotype	-0.91	0.13	-6.97	.000	-1.19	-0.64
3 N * chronotype	-0.96	0.13	-7.62	.000	-1.21	-0.71
4 N * chronotype	-1.16	0.26	-4.51	.000	-1.68	-0.65

In summary, mixed-effects models showed a three-way interaction effect: a higher number of consecutive morning shifts (*i.e.* 2 to 4) decreased mid-sleep deviations the later the chronotype, whereas increasing the number of successive night shifts (*i.e.* 2 to 4) enhanced mid-sleep deviations the earlier the chronotype (Fig. 4.6).

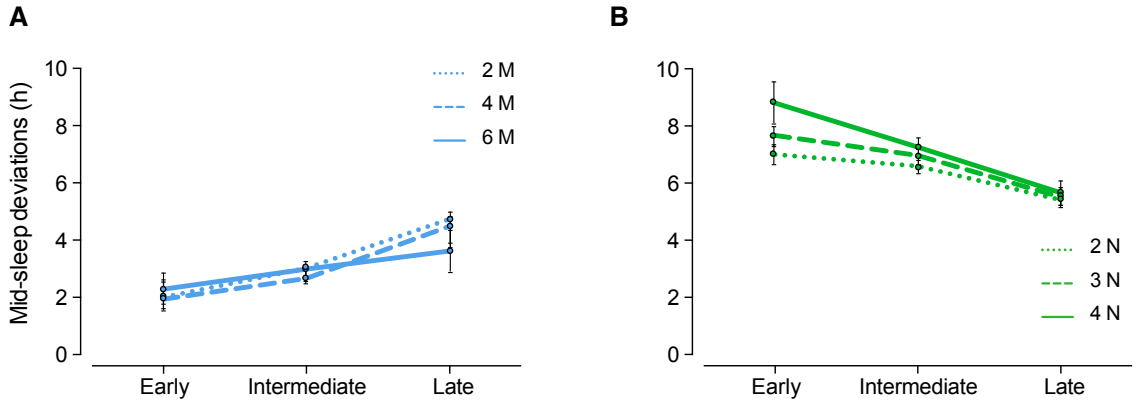

Figure 4.6. Mid-sleep deviations for morning **(A)** and night shift sequences **(B)** according to chronotype. Average values (± standard deviation) are shown. Please note: 1) Values for sequence of six morning shifts (panel A, straight blue line) were based on eleven participants only and model estimate was non-significant. Thus, the numeric decrease cannot be interpreted meaningfully. 2) Regression analyses were always based on individual data and grouped data (early, intermediate, late chronotype groups) are shown for illustrative purposes only. Cut-offs were chosen according to inter-quartile range of the sample (early chronotypes: $MSF^{E}_{sc} < 3:43$, n = 23; intermediate types: $3:43 \leq MSF^{E}_{sc} \leq 5:31$, n = 50; late types: $MSF^{E}_{sc} > 5:31$, n = 24).

4.4.4 Deriving predictions from statistical modelling

Using the unstandardised estimates from the mixed-effects models allows for predicting mid-sleep deviations on varying shift sequences. Here, six different exemplified sequences are modelled, each consisting of a block of six consecutive workdays in a forward rotation. For example, the sequence '222' in Figure 4.7 represents two morning shifts followed by two evening shifts and subsequently two night shifts. Values are estimated assuming different chronotypes, ranging from very early to very late ones (MSF^{E}_{sc} = 2:00 – 8:00 a.m.). Mid-sleep deviations are fairly similar across chronotypes for the '222'

sequence, as everyone has a 'beneficial' and a 'less beneficial' type of shift to work (*i.e.* morning shifts for late and night shifts for early types). Working a relatively high number of morning shifts and no night shifts (*i.e.* '600' and '420') enlarges the differences between chronotypes with late types showing drastically increased mid-sleep deviation values. Similarly, working no morning shifts but up to four night shifts in a row (*i.e.* '024' and '042') enhances mid-sleep deviations immensely for early types. Furthermore, differences in mid-sleep deviations according to sequence are greatest for early types as mid-sleep deviations are very low on morning shifts but extremely large when working at night. Late chronotypes in contrast show relatively small mid-sleep deviations on night shifts but not as small as early types on morning shift (*i.e.* the earliest chronotype of 2:00 a.m. shows a difference of 4.12 (h) between smallest and largest value, while this difference is only 2.21 (h) for the latest chronotype of 8:00 a.m.). Finally, model estimates were used to determine a cut-off value for MSF^{E}_{sc} that is 'late enough' to enable lower mid-sleep deviations on night shift sequences than on morning shift sequences. Calculations revealed that a shift worker would need to be as late as 7:00 a.m. to benefit from working at night compared with morning shifts (Fig. 4.7, red line).

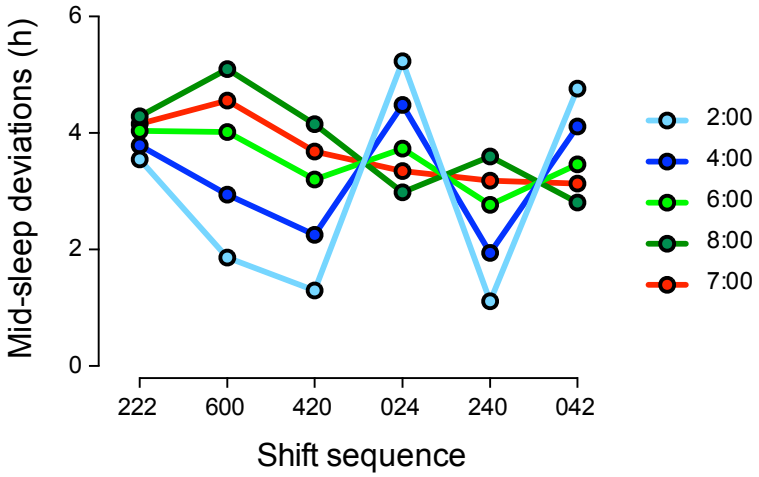

Figure 4.7. Model predictions for mid-sleep deviations on varying shift sequences according to chronotype. Shift sequences consist of six workdays with *n* morning, *n* evening, and *n* night shifts (*i.e.* '600' indicates six morning, zero evening, and zero night shifts). Values are modelled for different chronotypes (*i.e.* 2:00 (early type), 4:00 (intermediate), 6:00 and 8:00 a.m. (late types)). The red line represents the MSF^{E}_{sc} cut-off value of 7:00 a.m. when working night shifts involves less mid-sleep deviations than working morning shifts.

4.5 Discussion

Up to the author's knowledge, this is the first study providing detailed analyses of sleep-wake behaviour on single shift sequences taking into account individual internal time, *i.e.* chronotype. Sleep log data from 97 rotational shift workers in 7 different schedules were examined allowing for comparison of different numbers of consecutive (morning, evening, night) shifts. As outcome variables, differential sleep duration (difference in sleep duration between workdays and work-free days) and mid-sleep deviations (a measure for circadian disruption of sleep-wake cycle) were calculated revealing chronotype-specific effects.

Increasing the number of successive morning shifts from two to four had no impact on differential sleep duration, as both, sleep length on workdays and work-free days, remained unchanged. Yet, with a higher number of morning shifts, mid-sleep deviations decreased the later the

chronotype (by 13 minutes with each hour delayed). Only 11 participants worked the shift sequence of six successive morning shifts limiting its results' reliability. Working this sequence first seemed to reduce sleep deprivation and circadian misalignment the later the chronotype. However, further analyses revealed one participant potentially biasing results towards a positive effect. This participant (MSF^{E}_{sc} = 5:37) showed very short sleep duration on morning shifts, and also very short sleep on day off whilst indicating no use of alarm clock in the sleep log. Thus, the noticeably decreased sleep duration of 5 h on the first work-free day might itself reflect an adverse effect of a higher number of consecutive morning shifts in late chronotypes.

Increasing the number of consecutive night shifts from two to four also revealed chronotype-specific effects: sleep duration decreased after night shifts and increased on first work-free day for early types, indicating accumulated sleep debt. In contrast, night shift sleep lengthened and sleep on day off shortened the later the chronotype (reaching almost identical sleep durations). Additional calculations suggested that already a chronotype of ≥ 3:49 a.m. (German local time) might profit from an increased number of night shifts in terms of getting more sleep. Please note, that such cut-off values should be treated carefully and only with respect to a reference population as seasonal variations and geographical location are influencing factors for sleep-wake behaviour and chronotype.[18] A higher number of consecutive night shifts (*i.e.* 2 *vs.* 4) also reduced mid-sleep deviations the later the chronotype (by 15 minutes with each hour delayed) while drastically increasing the extent of mistimed sleep in early types.

Model estimates for different numbers of successive morning, evening, and night shifts were used to simulate various shift sequences each consisting of six consecutive workdays. Predicted values showed a chronotype cut-off of ≥ 7:00 a.m. (German local time) to potentially benefit from sequences comprising night shifts rather than sequences entailing morning shifts. In Germany, only ~7% of the population displays an MSF_{sc} later than 7:00 a.m.[15]

Again, such cut-off values should be interpreted and applied only relative to a reference population including information on season, longitude, and latitude.

Current European guidelines recommend that shift workers should generally not work more than three night shifts in a row. The data on sleep-wake behaviour presented here suggest that the number of consecutive shifts beneficial for an individual strongly depends on chronotype. Accordingly, early chronotypes seem to better avoid more than two successive night shifts whereas late chronotypes potentially profit from four night shifts worked in a row. Current research often compares consecutive night shifts with non-night shifts or days off,[19,20] and only few studies examine the impact of varying numbers of successive shifts revealing ambiguous results. In a pooled analysis, the risk of a negative occurrence (*i.e.* accident, injury) was approximately 6% higher on the second night, 17% higher on the third night, and 36% higher on the fourth night.[21] This finding was also true for morning shifts, although to a weaker extent: the average risk was about 2% higher on the second morning, 7% higher on the third morning, and 17% higher on the fourth morning shift than on the first day. A recent case-control study confirmed those results in an inpatient care staff sample where injury risk was almost 3-fold higher for three or more consecutive night shifts.[22] A laboratory study on simulated night work (0:00 – 8:00 a.m.) found that melatonin production decreased progressively over three consecutive night shifts.[23] Because light intensity was low (50 lux at eye level) and the decrease was progressive, authors concluded that direct melatonin suppression by exposure to light at night was probably not a significant factor. Barton and co-workers examined the question of an optimum number of night shifts and showed a clear positive, but indirect effect of consecutive night shifts on health and well-being as assessed with the Standard Shiftwork Index.[24] With more night shifts worked, sleep duration and sleep quality increased which were in turn strong predictors of self-reported psychological and physiological health. Several studies indicated better adaptation to night shifts on the fourth night shift as

demonstrated by improvements in cognitive performance at night.[25–27] A recent study by Chang and colleagues examined two independent samples of nurses working 2 and 4 consecutive night shifts, respectively.[28] Nurses were tested during the day after (2 *vs.* 4) night shifts applying a test battery of questionnaires and cognitive performance tests. Those working two successive night shifts showed poorer performance directly after the night shift and lacked learning effects on a visual attention test compared with nurses working four successive night shifts. The authors suggested that rotating night shifts too quickly might impede attention-related performance. Yet, they tested nurses at the end of a night shift block not examining what happened after the second night shift in nurses regularly working four nights in a row. It would have been interesting to study in a within-subject design whether increases in performance were due to a chronic adaptation effect (*i.e.* no difference in performance after second and fourth night), or due to an acute effect by lengthening the night shift block (*i.e.* worse performance after second than fourth night). Overall, literature suggests that extended blocks of consecutive shifts (≥ 4) foster adaptation, increase sleep duration and enhance cognitive performance,[25–29] whereas fast rotations (< 4 consecutive shifts) appear to reduce accumulation of sleep debt, decrease on-shift fatigue and sleepiness, and lower risk of injuries;[21,30,31] results that are somewhat contradictory.

A possible explanation might be inter-individual variation in the ability to tolerate circadian misalignment and inappropriate phasing (trying to stay awake or go to sleep when the circadian clock dictates otherwise).[29] Workers who prefer night shifts in prolonged blocks may be able to overcome circadian misalignment in a relatively short time while workers who opt for stand-alone night shifts may have more difficulties overcoming those problems. Chronotype as well as age appear reasonable candidates for such individual differences. Härmä et al. accompanied the change from a continuous backward rotation (four consecutive shifts of morning, night, and evening

work) to a very quickly forward rotating schedule (one shift of morning, evening, night work) and found positive effects on sleep-wake behaviour, sleepiness, and psychomotor performance, which were more pronounced in older shift workers.[32] Given that chronotype advances with age,[33] the here presented results of early types benefitting most from short blocks of consecutive shifts agree with their findings.

Several limitations need to be mentioned. First, sleep log data constitute subjective assessments susceptible to recall errors and perception biases. Yet, comparison with available actimetry data (as some participants filled out sleep logs and wore actimeters) revealed good congruence confirming validity of subjective sleep log entries (data not shown). Second, shift sequences were determined as blocks of consecutive workdays framed by days off, following a distinct, non-explorative, a-priori defined approach. This approach was chosen to enhance comparability between sequences assuming 'wash-out' effects by work-free days in-between. Sequences might be defined otherwise in future studies; yet, similar results would be expected. Furthermore, slow rotations usually involve longer periods (*e.g.,* 3 weeks) on each shift before a change is instituted. Thus, sequences analysed here are essentially quickly rotating sequences and future studies are warranted to compare those with blocks of more than 6 consecutive shifts. Third, shift sequences involving three night shifts and six morning shifts should be interpreted with carefulness as sample size was limited (n_{3N} = 19, n_{6M} = 11). Fourth, only sleep timing and duration was assessed not allowing for ascertaining what happened during sleep (*e.g.,* ultradian rhythmicity of NREM/REM sleep cycles). Last, sleep-wake behaviour is one of many factors in shift work. A specific number of night (or morning) shifts might be beneficial in terms of sleep but detrimental with regards to other aspects, such as social and family life, alertness and fatigue during working time, financial remuneration, production requirements, etc. Further research is needed to clarify interactions between those variables and number of consecutive shifts.

Most importantly, future studies should include individual chronotype as not doing so might average out meaningful effects.

In summary, the results presented here demonstrate a clear chronotype-effect on number of consecutive shift beneficial for an individual's sleep-wake behaviour. Translating those findings into real-world applications for the design of shift schedules implies both, challenges and potentials. On one hand, considering individual characteristics, such as chronotype, will increase complexity not only of shift schedules, but also of logistics, personnel management, wage and salary administration, occupational medical care, and so forth. On the other hand, following the principle "one size fits all" in order to minimise negative impacts of shift work may have reached its limit, and individualisation of schedules using chronotype differences appears promising to further optimise shift systems by matching individual temporal niches (internal time) with occupational ones (external time). In view of an increasing percentage of people working in shifts and the associated health burden, managing higher complexity for the sake of healthier schedules seems worth thinking about.

4.6 Appendix

4.6.1 Sleep duration between workdays and work-free days on evening shift sequences

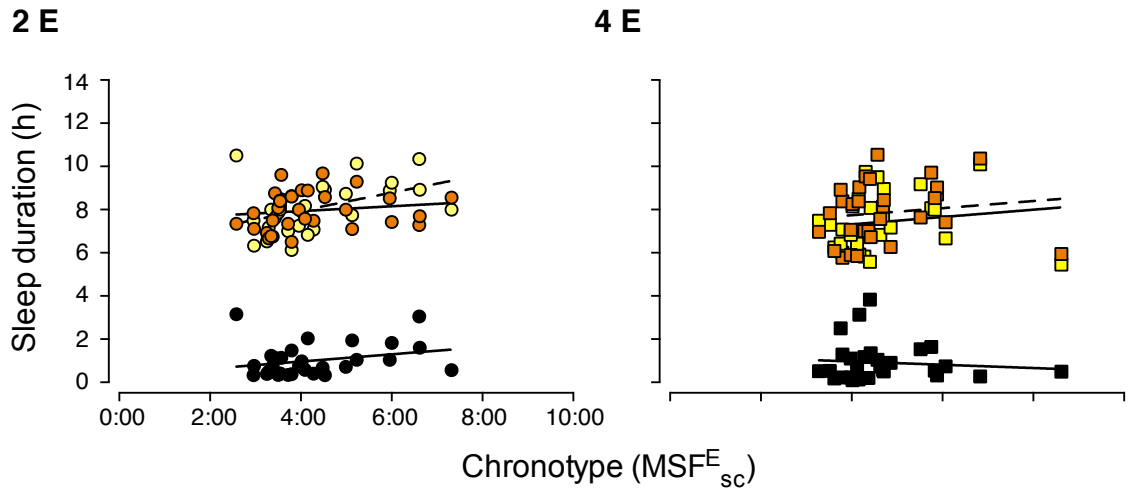

Figure A4.1. Analyses of sleep duration on evening shift sequences (2 and 4 E). Black symbols = difference in sleep duration on workdays and work-free days. Orange symbols = averaged sleep duration on consecutive evening shifts. Yellow symbols = sleep duration on first work-free day after evening shifts (yellow symbols). Straight line = trend on workdays, dotted line = trend on work-free days.

4.6.2 Mid-sleep deviations on evening shift sequences

Table A4.1. Mixed-effects regression model for evening shift sequences (2 and 4 consecutive evening shifts (E)). Please note that none of the covariates age, gender, body mass index, shift schedule, and overall sleep duration contributed significantly and were therefore removed from the model. Estimate = unstandardised regression coefficient. Se = standard error. CI = confidence interval. S1 / S2 / S3 = shift schedules from different study sites (see 4.3.1).

Variable	Estimate	Se	t	P > | t |	CI (95%)	
					Lower bound	Upper bound
Intercept	1.80	1.63	1.11	.268	-1.39	4.98
Chronotype (MSF^{E}_{sc})	0.33	0.37	0.87	.387	-0.42	1.09
2 E	-0.36	0.82	-0.44	.659	-1.96	1.24
4 E	-0.35	0.94	-0.37	.709	-2.19	1.49
2 E * chronotype	-0.26	0.18	-1.43	.154	-0.62	0.10
4 E * chronotype	-0.39	0.19	-1.99	.056	-0.78	-0.01

5 References

5.1 References General Introduction

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7 Eidesstattliche Versicherung

Fischer, Dorothee

_____

Name, Vorname

Ich erkläre hiermit an Eides statt,

dass ich die vorliegende Dissertation mit dem Thema

Of ‘Islands and Pancakes’: The Shape of Sleep in Shift Work

selbständig verfasst, mich außer der angegebenen keiner weiteren Hilfsmittel bedient und alle Erkenntnisse, die aus dem Schrifttum ganz oder annähernd übernommen sind, als solche kenntlich gemacht und nach ihrer Herkunft unter Bezeichnung der Fundstelle einzeln nachgewiesen habe.

Ich erkläre des Weiteren, dass die hier vorgelegte Dissertation nicht in gleicher oder in ähnlicher Form bei einer anderen Stelle zur Erlangung eines akademischen Grades eingereicht wurde.

_____

Ort, Datum

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Unterschrift Doktorandin/Doktorand

8 Deutsche Zusammenfassung

Praktisch alle Organismen dieser Erde weisen eine innere Uhr auf, die sie in die Lage versetzt, auf äußere Veränderungen nicht nur zu reagieren, sondern diese zu antizipieren und entsprechende Anpassungen vorzunehmen. Hierfür ist der Wechsel von Tag und Nacht das zuverlässigste und dominanteste Signal für Tages- und Jahreszeit (sog. ‚Zeitgeber'). In modernen Zeiten wird dieser natürliche Rhythmus herausgefordert durch Arbeits- und Familienanforderungen, Freizeitgestaltung und Lebensstil. Künstliches Licht ermöglicht es uns rund um die Uhr zu arbeiten und sprichwörtlich die ‚Nacht zum Tage' zu machen. Das Missverhältnis von innerer, biologischer Zeit (z.B. Schlaf-Wach-Zyklus) und äußerer, sozialer Zeit (z.B. Arbeitszeit) wird als *circadiane Disruption* bezeichnet und begünstigt potentiell Entstehung und Verlauf diverser Krankheiten, wie Herz-Kreislauf-Erkrankungen, metabolische und psychiatrische Störungen. Am deutlichsten wird das Missverhältnis von ‚Körperzeit' und Arbeitszeit am Beispiel der Schichtarbeit: Schichtarbeiter – ob in permanenten oder rotierenden Modellen – essen, trinken, schlafen und sind aktiv zu Zeiten, an denen ihr Körper eigentlich auf das Gegenteil eingestellt ist. Welche Zeiten nun für einen einzelnen Menschen von Vor- oder Nachteil für die Gesundheit sind, darüber entscheidet auch der sogenannte *Chronotyp*. Der individuelle Chronotyp repräsentiert die circadiane Phasenlage der inneren Uhr, d.h. die zeitliche Einbettung biologischer Rhythmen in den gegebenen Tag-Nacht-Verlauf. Die Unterschiede dieser zeitlichen Einbettung können enorm sein zwischen Individuen und im Falle des Schlaf-Wach-Verhaltens bis zu 12 Stunden betragen: wenn eine Person aufsteht, geht die andere gerade zu Bett. Diese Spanne gilt auch dann (und dann erst recht), wenn keine äußeren Restriktionen Einfluss ausüben, wie Wecker oder soziale Veranstaltungen. Aus der Existenz solcher inter-individuellen Unterschiede der inneren Uhr des Menschen lässt sich schlussfolgern, dass es auch

individuelle Unterschiede gibt, welche Arbeitszeiten potentiell belastend und gesundheitlich problematisch sind und damit eine circadiane Disruption darstellen. Schichtarbeit mit circadianer Disruption wurde 2007 von der Internationalen Agentur für Krebsforschung (IARC) als wahrscheinlich krebserzeugend für den Menschen eingestuft. Obwohl damit angenommen wird, dass circadiane Disruption der kausale Zusammenhang zwischen Schichtarbeit und Krankheiten sei, wurde keine klare Definition von der IARC vorgenommen. Neben der theoretischen Einbettung fehlen auch quantitative Messungen, wodurch die systematische Untersuchung der Ursachen und Folgen von circadianer Disruption erheblich erschwert wird. Eine solche Quantifizierung, wie auch immer geartet, muss jedoch die innere Uhr und den individuellen Chronotyp des Menschen berücksichtigen, da andernfalls signifikante Effekte über- oder unterschätzt würden.

Die vorliegende Dissertation beschäftigt sich mit der Erfassung und Untersuchung circadianer Disruption im Kontext reeller Schichtarbeit. In **Projekt Eins** wird das Schlaf-Wach-Verhalten von 35 Schichtarbeitern in einem 12-h Schichtmodell untersucht. Die Ergebnisse verdeutlichen, wie wichtig der individuelle Chronotyp und die spezifische Schichtabfolge für circadiane Disruption und Schlafdauer sind und legen den Grundstein für das nächste Projekt. In **Projekt Zwei** wird eine neue Methode vorgestellt zur Quantifizierung circadianer Disruption des Schlaf-Wach-Verhaltens. Die Methode, genannt ‚mid-sleep deviations', integriert zwei entscheidende Aspekte: Innenzeit und vorangegangene Schlafepisode. Die Anwendung dieses Ansatzes offenbart eine distinkte und neuartige Geometrie im Schlaf-Wach-Verhalten von 53 Schichtarbeitern. In **Projekt Drei** werden bestehende Leitlinien zur Gestaltung von Nacht- und Schichtarbeit mithilfe der ‚mid-sleep deviations' neu untersucht. Statistische Analysen (‚mixed effects models') der Daten von 97 Schichtarbeitern aus sieben verschiedenen Schichtsystemen zeigen, dass die Anzahl aufeinanderfolgender (Nacht-)Schichten, die bislang als empfehlenswert betrachtet wird, stark vom individuellen Chronotyp

abhängt. Im Folgenden ist jedes der drei Projekte noch einmal ausführlicher dargestellt.

Projekt Eins

„Schlaf-Wach-Verhalten in einem rotierenden 12-h Schichtsystem: Die Bedeutung von Chronotyp und Schichtabfolge“

Die Studie untersucht unter Berücksichtigung des individuellen Chronotyps das Schlaf-Wach-Verhalten von Schichtarbeitern in einem schnell-vorwärts rotierenden 12-h Schichtsystem (Tagschicht: 6:00 – 18:00, 24 Stunden frei, Nachtschicht: 18:00 – 6:00, 48 Stunden frei). Fünfunddreißig Schichtarbeiter des deutschen Chemie-Konzerns BASF füllten den Münchner Chronotyp Fragebogen für Schichtarbeiter aus und trugen kontinuierlich für zwei Wochen ein Aktimetrie-Messgerät am Handgelenk. Schlaf- und Nickerchendauer, sozialer Jetlag (ein Maß für circadiane Disruption) und circadiane Phasenmarker (Schlafmitte, ‚Aktivitätsschwerpunkt') wurden aus den Aktimetrie-Daten berechnet. Je früher der Chronotyp eines Mitarbeiters, desto höher der soziale Jetlag, kürzer die Schlafdauer und länger das vorausgehende Nickerchen (bis zu 3h) an Nachtschichttagen. Wurden Nickerchen und Hauptschlaf-Dauer zusammen betrachtet, schliefen alle Chronotypen ungefähr gleich lang. Alle Mitarbeiter, jedoch insbesondere späte Chronotypen, schliefen am längsten zwischen Tagschicht und Nachtschicht (~9h im Durchschnitt). Der untersuchte 12-h Schichtenplan der BASF stellt insofern ein besonderes Modell dar, als Ruhephasen von mindestens 24 Stunden nach jeder Arbeitsschicht vorgegeben sind. Dadurch können die Schichtarbeiter ein aufkommendes Schlafdefizit unverzüglich nach jeder Schicht kompensieren. In vorherigen Studien wurden hinsichtlich verschiedener Gesundheitsvariablen keine Unterschiede zwischen der Tag- und der Schicht-arbeitenden Belegschaft der BASF gefunden. Die spezifische

Schichtabfolge des BASF Modells könnte über das Schlaf-Wach-Verhalten potentiell zur beobachteten Krankheitsminimierung beitragen.

Projekt Zwei

„Mid-Sleep Deviation: Quantifizierung und Visualisierung circadianer Disruption des Schlaf-Wach-Zyklus“

Circadiane Disruption ist ein potentieller Mechanismus für negative Auswirkungen auf die Gesundheit im Kontext von Schichtarbeit. Obwohl der Begriff weit verbreitet ist, existieren nur wenige Definitionen und Quantifizierungen, wodurch die systematische Untersuchung von Ursachen und Folgen der circadianen Disruption erschwert wird. Eine kürzlich erschienene Studie konnte zeigen, dass ‚Schlaf zur falschen Zeit' die circadiane Regulation des menschlichen Transkriptoms stark beeinträchtigt. In Projekt Zwei wird eine neue Methode vorgestellt, die sogenannten ‚mid-sleep deviations', die das Ausmaß eines solchen Schlafes ‚zur Unzeit' in eine numerische Größe überführt. Aktimetrie-Daten von 53 Schichtarbeitern aus vier verschiedenen vorwärts-rotierenden 3-Schicht-Systemen (55% weiblich, 35 ± 10 Jahre, Bodymass Index 26 ± 5 kg/m^2) wurden analysiert. Der individuelle Chronotyp wurde auf Aktimetrie-Basis bestimmt und die täglichen Schlafmitten (als circadiane Marker des Schlaf-Wach-Verhaltens) über einen Zeitraum von zwei bis vier Wochen extrahiert. Die Methode der ‚mid-sleep deviations' integriert zwei wichtige Aspekte des Schlafes: Innenzeit (‚Chronotyp') und Schlafhistorie (‚vorangegangene Schlafepisode'). Beide Aspekte werden in Relation zueinander gesetzt und enthüllen so eine charakteristische und chronotyp-spezifische Geometrie des Schlaf-Wach-Verhaltens in Schichtarbeit. Um diese Geometrie weiter zu veranschaulichen, wurde die Dichte der Datenpunkte berechnet und mit farbigen Konturlinien visualisiert (‚density plots'). Sowohl Berechnung als auch grafische

Darstellung der ‚mid-sleep deviations' deuten darauf hin, dass späte Chronotypen ein variableres Schlaf-Wach-Verhalten aufweisen als frühe Chronotypen, statistisch unabhängig von demografischen Variablen, Schichtrotation und durchschnittlicher Schlafdauer. Der Vergleich mit zwei existenten Messungen circadianer Disruption (‚inter-daily stability' und ‚behaviorales entrainment') zeigte eine gute, generelle Übereinstimmung; jedoch legen weitere Analysen nahe, dass ‚mid-sleep deviations' zusätzliche Informationen über die Störung von Schlaf-Wach-Zyklen bereithalten. Die Methode der ‚mid-sleep deviations' als Maß für die circadiane Disruption des Schlaf-Wach-Verhaltens dient potentiell dazu, die Rolle von gestörten Schlaf-Wach-Rhythmen für Krankheitsentstehung und –verlauf auf individueller Basis zu bestimmen.

Projekt Drei

„Überprüfung gegenwärtiger Leitlinien zu Nacht- und Schichtarbeit"

Aktuelle europäische Leitlinien zu Nacht- und Schichtarbeit umfassen mehrere Empfehlungen für die Gestaltung von Schichtenplänen, die wiederum darauf abzielen, negative Auswirkungen für Gesundheit, Sicherheit und Sozialleben zu verringern. Entsprechend sollten u.A. langsame Rotationen und mehr als drei aufeinanderfolgende Nachtschichten vermieden werden. Bislang fehlen detaillierte Analysen dieser Leitlinien, die individuelle Merkmale wie Chronotyp explizit berücksichtigen. In der vorliegenden Studie werden darum die Effekte einzelner Schichtabfolgen im Lichte des individuellen Chronotyps bewertet. Siebenundneunzig Schichtarbeiter aus sieben verschiedenen vorwärts-rotierenden Schichtsystemen (31% weiblich, 36 ± 10 Jahre, Bodymass Index 26 ± 5 kg/m^2) führten Schlaftagebuch über einen Zeitraum von vier bis zwölf Wochen. Aus diesen Angaben wurden die Schlafdauer an Arbeits- und freien Tagen sowie ‚mid-sleep deviations', ein in Projekt Zwei entwickeltes Maß für

die circadiane Disruption des Schlaf-Wach-Verhaltens, berechnet. In Regressionsmodellen mit gemischten Effekten (‚mixed effects models') wurden unterschiedliche Anzahlen konsekutiver (Früh-, Spät-, Nacht-) Schichten statistisch analysiert. Die Ergebnisse zeigen einen Interaktionseffekt zwischen Chronotyp, Art und Anzahl der Schichten: mit einer größeren Zahl aufeinanderfolgender Nachtschichten (z.B. 2 *vs.* 4 Nachtschichten) nahmen sowohl ‚mid-sleep deviations' als auch Schlafdeprivation für frühe Chronotypen zu, wohingegen sich beide Variablen bei späten Chronotypen verringerten. Ein gegenteiliger Effekt wurde für Frühschichten beobachtet. Diese Befunde legen nahe, dass die Anzahl konsekutiver Schichten, die aus ergonomischer Sicht als empfehlenswert betrachtet werden kann, stark vom individuellen Chronotyp abhängt. Die Ergebnisse der vorliegenden Studie dienen dazu, bestehende Schichtarbeits-Leitlinien insbesondere im Hinblick auf die biologische Innenzeit zu evaluieren und zu optimieren.