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Of larks and hearts — morningness/eveningness, heart rate variability and cardiovascular stress response at different times of day

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ABSTRACT

Inter-individual differences in the circadian period of physical and mental functions can be described on the dimension of morningness/eveningness. Previous findings support the assumption that eveningness is related to greater impulsivity and susceptibility to stress than morningness. Heart rate variability (HRV) serves as a physiological correlate of self- and emotional regulation and has not yet been investigated in relation to chronotypes. The study explores differences in HRV and other cardiovascular measures in morning- and evening-types at rest and under stress at different times of day (8–11 a.m. or 4–7 p.m.). Students ($N = 471$) were screened for chronotype and $n = 55$ females (27 morning- and 28 evening-types) were recruited for testing. These participants performed a mental arithmetic task while heart rate (HR) and blood pressure (BP) were recorded. Spectral components and a time-domain measure of HRV were calculated on HR data from resting and mental stress periods. Evening-types had significantly higher HR and systolic BP, but lower HRV than morning-types both at baseline and during stress. Stress induced in the evening had a significantly stronger impact on absolute and baseline corrected physiological measures in both chronotypes. The interaction of chronotype and testing time did not reach the level of significance for any of the dependent variables. The enhanced physiological arousal in evening-types might contribute to increased vulnerability to psychological distress. Hence, previous behavioral findings are supported by the physiological data of this study.

1. Introduction

In humans and other mammals, many physiological processes, such as the regulation of body temperature or levels of cortisol [1] and melatonin [2] as well as mental processes (e.g. alertness, working memory, or measures of fluid intelligence) [3,4] are affected by circadian rhythms. Individual differences in these chronobiological rhythms can be summarized under the concept of morningness/eveningness (M/E). Morningness and eveningness can be considered two poles on a scale, on which a person's chronotype can be defined. Depending on the diurnal preference, the phase position and period of the circadian rhythms, people may either be morning- or evening-orientated (M- or E-type) or, like most, be a neutral chronotype [5]. In twin studies, between 44 and 54% of the total variance of M/E could be explained by genetic factors [6,7]. Chronotypes differ with regards to their sleeping behavior [8], personality [9], mental health [10], smoking habits [11] and even their sense of humor [12].

1.1. M/E and self-regulation

Present findings indicate that eveningness is related to a greater susceptibility to stress [13]. E-types are less emotionally stable than M-types [14,15] and have a higher prevalence of psychosomatic symptoms [16], which supports the idea of eveningness being related to increased reactivity to stress, reduced coping abilities, or both. Consequently, one may assume that M/E could have an impact on general well-being, in particular when exposed to chronic stress. Recently, it was shown that morningness correlated positively with life satisfaction [17], and that the relationship was negative between morningness and stress characterized by chronic non-specific arousal [18]. Studying the association of eveningness and Type A personality has led to contradictory results, such that a positive [16] and a negative [19] relationship could be found.

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Studies in adolescents have shown a higher prevalence of behavioral and emotional problems, and habitual substance abuse in evening orientated teenagers [20,21]. Positive relationships have also been found between eveningness and depression [22], the amount of percieved self- and proxy-related problems [23], as well as eating disorders [24,25]. Furthermore, there is evidence for E-types having more situation specific problems than M-types in their capabilities of inhibiting, interrupting and adapting their current behavior [16]. Moreover, eveningness correlated positively with novelty seeking (a personality trait including aspects of impulsivity) and negatively with persistence [26]. The primary reason for the reduced self-regulatory capacity and emotional stability in E-types compared to other chronotypes remains unclear. Buschkens and colleagues [18] hypothesized that a less adaptable central nervous system in E-types might be responsible for their reduced regulatory capacity and that their impaired well-being may in part result from elevated arousal.

1.2. M/E and HRV

HRV is defined as the variability of the time elapsing between two successive heartbeats and serves as an indicator of a person's regulatory abilities regarding physiological, affective and cognitive processes [27]. According to the model of neurovisceral integration [28], prefrontal cortical regions inhibit subcortical structures such as the amygdala. The inhibition of the amygdala is associated with more parasympathetic (suppressive) and less sympathetic (activating) influence on heart rate. It is widely accepted that prefrontal cortical activity is the main correlate of executive functions (e.g. planning, initiating, monitoring and inhibiting behavior, impulse control, priority setting and emotional regulation) [27]. The more a task challenges these executive functions, the more inhibition is required by the prefrontal cortex. Pronounced executive functioning is accompanied by higher parasympathetic influence, which decreases HR and increases HRV. Less advanced executive functioning is accompanied by sympathetic dominance and related to higher HR and lower HRV. These interactions enable individuals to respond to and adapt their level of activation appropriately to environmental demands. Therefore, HRV can be interpreted as an index of an individual's ability to modify and regulate behavior and emotions in a quick, flexible and effective manner [29]. As opposed to the link between increased HRV and effective self-regulation, decreased HRV has been found to be associated with higher negative emotional arousal in response to stress and maladaptive coping strategies [30]. Accordingly, sleep disturbances [31], general anxiety disorder [32], panic disorder [33], and depression [34] were related to diminished vagally mediated HRV.

1.3. M/E and cardiac stress response

In a comprehensive review of the literature by Cavallera and Giudici [14], only two studies on M/E included the monitoring of cardiovascular stress response. Nebel and colleagues [35] confronted their male subjects with mental and physical stressors in the morning (7:30 a.m.) and at noon (12:30 p.m.). They found a significant interaction of M/E and testing time for heart rate (HR) and rate by pressure product (HR × systolic blood pressure, RPP) only during mental stress (a math test and a Stroop color word task). This interaction was evident for absolute levels and for baseline-corrected change scores. M-types exhibited higher HR and RPP during the morning session, whereas E-types had higher levels during the afternoon session.

The second study by Willis and colleagues [36] tried to replicate these findings in a larger mixed sample and found that E-types had significantly higher HR and RPP in the afternoon (1–2 p.m.) than in the morning (8–9 a.m.), at rest and in response to stress, but the interaction effect was not significant for change scores. In neither study, M/E or time of day has a significant main effect on cardiovascular parameters. Women had higher HR scores and lower systolic BP (SBP) than men, at baseline and during stress.

1.4. Study design and hypotheses

The aim of the current study was to investigate the relationship between M/E and cardiovascular responses to mental stress at different times of day. In addition to cardiovascular parameters, i.e. HR and BP, we focused on HRV because it may mediate and explain findings on psychological and behavioral differences in relation to M/E. Results from several studies on cognitive processes, such as memory performance [37,38] or attention [39], indicate that the peak of performance for E-types does not occur before 4 p.m. Thus, we chose a time window between 4 and 7 p.m., which is considerably later than in previous studies [35,36]. To avoid habituation to the laboratory stressor we chose a between-subjects instead of a within-subjects design. Following Willis and colleagues [36] we used a mental stressor as this has proven more appropriate than physical stress [35].

We hypothesized that (I) E-types would show reduced HRV and increased HR and BP levels compared to M-types at baseline. Further, we predicted (II) cardiovascular reactivity to stress (corrected for baseline levels) to be higher in E- than in M-types and (III) an interaction of M/E and testing time for cardiovascular reactivity to mental stress.

2. Methods

2.1. Design

The study followed a 2 (trial) × 2 (chronotype) × 2 (testing time) design with chronotype (morning vs. evening) and testing time (8–11 a.m. vs. 4–7 p.m.) as between-subject factors and trial (baseline vs. stress) as within-subject factor.

2.2. Participants

We screened online N = 471 students of the University of Würzburg with the German version of the Morningness–Eveningness Questionnaire (D-MEQ, see Section 2.3). Student councils of the University of Würzburg were contacted via e-mail and asked to send the Internet link to the voluntary online survey over the student councils' mailing lists. Screened participants were on average $M = 23.18$ years old ($SD = 4.00$, range 16–61), and 76.9% ($n = 362$) were female. Mean D-MEQ score was $M = 50.11$ ($SD = 7.24$) and women had significantly higher values than men ($t_{(469)} = 2.45$, $p < .05$). Since the majority of the participants were female, we decided to recruit only women for the experiment. Individuals in the upper and lower 20% of the D-MEQ distribution (D-MEQ scores ≤ 44 for E-type and ≥ 56

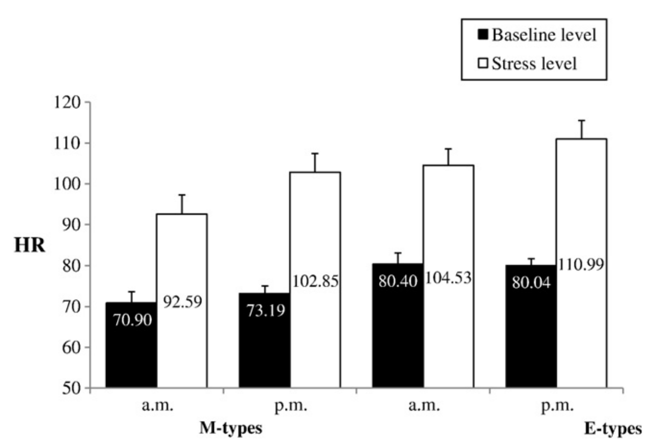

Fig. 1. HR at baseline and stress level. Error bars indicate standard error.

for M-type), who had agreed to take part in the experimental session, were contacted via e-mail to schedule an appointment.

A sample of $N=63$ was investigated in the laboratory, but eight subjects were excluded due to (a) taking medication other than contraceptives ($n=3$), (b) a Body Mass Index (BMI) > 30 kg/m^2 ($n=3$) and (c) baseline HR > 100 bpm ($n=2$), leaving a final sample of $N=55$ participants with an age range of 19–30 years ($M=23.04$, $SD=2.37$). These participants were randomly assigned to the following experimental conditions: (a) M-types tested in the morning ($n=14$), (b) M-types tested in the evening ($n=13$), (c) E-types tested in the morning ($n=14$) and (d) E-types tested in the evening ($n=14$).

The single experimental sessions were conducted in a light- and temperature-controlled psychophysiological laboratory with participants attending individually. All participants gave written informed consent prior to taking part in the experiment. The experiment was conducted in accordance with standard ethical guidelines as defined by the Declaration of Helsinki (World Medical Association) and the European Council's Convention for the Protection of Human Rights and Dignity of the Human Being with regard to the Application of Biology and Medicine (Convention on Human Rights and Biomedicine). Participants were equally financially reimbursed or received course credits for participation.

2.3. Questionnaires

2.3.1. Morningness–Eveningness Questionnaire (MEQ) [40]

To determine diurnal preference in our subjects, we used the validated German version of the MEQ (D-MEQ) [41]. Classifications to one of the following categories are possible[1]: definite (16–30) or moderate E-type (31–41), neutral type (42–58), moderate (59–69) or definite M-type (70–86). D-MEQ scores and melatonin onset as a physiological measure of the circadian phase correlate significantly [41], supporting the validity of the questionnaire. Retest reliability ($r=.97$) is very good [41]. Higher values indicate more morningness.

2.3.2. Pittsburgh Sleep Quality Index (PSQI) [42]

As sleep has been shown to be related to both M/E [8] and HRV [31], we used this 4-week-retrospective instrument (Cronbach's $\alpha=.83$) to control for sleep quality. The scale contains a subjective rating of seven sleep-related categories: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medication and daytime dysfunction. Total PSQI-Scores can range from 0 to 21 with higher scores indicating a more disturbed sleep. A cut-off value of 5 distinguishes reliably good and poor sleepers [42].

2.3.3. Global Vigor and Affect scale (GVA) [43]

The GVA was used to control for participants' vigor and emotional state as this may impact their reactivity to stress. It consists of eight visual analog scales, on which subjects are asked to rate their actual state of alertness, sadness, tension, effort, happiness, weariness, calmness and sleepiness. Scores for each scale range from 0 to 100 with higher scores indicating stronger expression of the respective state. Scales can be assigned to the two dimensions Global Vigor and Global Affect. The GVA has been used successfully in several studies on alertness or sleep [5,44,45].

2.3.4. Stress coping inventory (SVF) [46]

A short version of this questionnaire (SVF78) was used to identify possible differences in coping with stress. Each item describes a possible response to a stressor and subjects are asked to indicate on a 5 point Likert scale to what extent they use the proposed strategy. Coping responses are categorized as positive/adaptive or negative/non-adaptive coping strategies and averaged to derive final scores for the two dimensions (Cronbach's $\alpha=.89/.94$).

2.3.5. Subjective stress rating

To evaluate subjective stress after each trial, subjects indicated on a scale from 0 to 10, how stressed they felt at this moment.

2.4. Psychophysiological measures

2.4.1. Heart rate

A Polar watch (*Polar RS 800 CX*, Polar Electro Oy, Kempele, Finland) recorded HR during the experiment, with a sampling rate of 1000 Hz. This method has previously been deployed successfully to determine HR and derive HRV from these recordings [47–49].

2.4.2. Blood pressure

Systolic (SBP) and diastolic BP (DBP) were recorded from the brachial artery of the non-dominant arm using a digital blood pressure monitor (OMRON M10-IT, Omron Medizintechnik, Mannheim, Germany).

2.5. Stress induction

Stress was induced using a mental arithmetic task. The task was adapted from the *Trier Social Stress Test* [50], one of the most widely used mental stress protocols [51]. While the task itself was not changed, we had only one observer (instead of two) and did not use the white lab coat as this would have been a very unusual and probably non-credible procedure compared to other experiments conducted at our department.

The task involves counting backwards from a four-digit number as quickly as possible by subtracting the figure 13 for a period of 5 min. During the task an observer was present who was introduced by the experimenter as specifically trained in recognizing if subjects were really doing their best. Additionally, subjects were instructed to look straight into a video camera placed next to the experimenter and her colleague. After each wrong answer the observer told the participant to start again at the very beginning.

2.6. Experimental procedure

Participants were instructed to refrain from caffeine, alcohol and physical activity for at least 1 h prior to the experiment. Nicotine consumption was not restricted to avoid withdrawal symptoms. Each experimental session began with the assessment of demographic variables (self-reported height, weight, sleep duration over the preceding night, the frequency of physical activity, and caffeine and nicotine consumption) and the presentation of the *GVA*. Then subjects were asked to put on the chest strap of the Polar watch and the blood pressure arm cuff for the baseline recording of the physiological data. During the 10 min baseline trial, subjects rested in a chair and wore earphones for sound insulation and a sleep mask to avoid visual distraction. In the second half of the baseline phase, the BP-measurement started with the cuff inflating automatically every 120 s for three times. After completion of the baseline trial, participants were asked for a subjective rating of stress and to fill in the *PSQI*. Next, the mental arithmetic task followed for a period of 5 min, while HR and BP were recorded. Again, subjects were asked to rate perceived stress and to fill out the *SVF*. Finally, subjects were debriefed.

2.7. Data preparation

In accordance with recommended standards [52], the second half (5 min) of the 10 min baseline trial and the 5 min of mental

[1] The percentile criterion applied in the present study extends the original MEQ-categories for moderate E- and M-types by ± 3 (see 2.2.).

arithmetic were used for HR analysis with *Kubios HRV 2.0* [53]. Trend components were removed with the smoothness priors method ($\lambda = 500$). Heartbeat intervals were visually scanned by the experimenter and corrected for artifacts using the default settings of the program.

2.7.1. HRV

We calculated a measure of the time domain, namely the Root Mean Square of Successive Differences (RMSSD), indicating the variability of time elapsing between two consecutive R waves in the electrocardiogram. RMSSD was preferred to other time domain parameters because of better statistical properties [52]. Higher values indicate higher HRV.

2.7.2. Sympathovagal balance

Besides time domain measures, frequency components can be analyzed which reflect parasympathetic and sympathetic influence on the sino-artial node in a more distinguished manner. Low frequency components (0.04–0.15 Hz, reflecting sympathetic and parasympathetic influences on HR) and high frequency components (0.15–0.4 Hz, reflecting parasympathetic influence on HR) of the frequency spectrum were determined by Fast Fourier Transformation. Their ratio (LF/HF) was calculated as an index of sympathovagal balance with lower scores indicating parasympathetic dominance.

RMSSD and LF/HF were log-transformed because of skewed distributions.

2.7.3. Blood pressure

Mean BP values were calculated for each of the three 120 seconds time windows during baseline and mental arithmetic and averaged for each trial.

Change scores were generated for all dependent variables by subtracting baseline-trial- from stress-trial-values. The change scores express the differences between the two trials and thus, a baseline-corrected measure of the stress response.

3. Results

3.1. Participant characteristics

Participant characteristics broken down by groups are summarized in Table 1. In line with previous results of other groups [11,54], we found significantly more smokers among E-types,[2] while M-types exercised more frequently[2], although absolute numbers of smokers and regular exercisers were small across both groups. No significant group differences were found for age, BMI, caffeine consumption, intake of contraceptives or the amount of sleep the night before testing.

3.1.1. PSQI

E-types had a significantly higher PSQI score than M-types, indicating that eveningness was related to lower sleep quality. Significantly more E-types ($n = 21$, 75.00%) than M-types ($n = 5$, 18.52%) were above the cut-off score of the PSQI ($X^2 = 17.59$, $p < .00$), indicating more sleep problems in E-types.

3.1.2. GVA

Global Vigor was significantly higher for M-types than for E-types but did not differ between times of day. No significant differences were found between groups and times of day on the Global Affect Scale. None of the interactions were statistically significant.

[2] In addition to the reported results, all analyses on HR, BP, HRV and sympathovagal balance were conducted with physical activity and smoking status as covariates. None of the two variables had a significant impact on the dependent measures (all $F_{(df)} < 1.85$, *ns.*).

Table 1
Means, frequencies and test statistic of participant characteristics.

	M-types	E-types	Test statistic
Age *M (SD)*	22.96 (2.19)	23.11 (3.20)	$t_{(47.89)} = -.20$, *ns.*
Smokers *n* (%)			
Yes	2 (7.41)	8 (28.57)	$\chi^2 = 4.14$, $p < .05$
No	25 (92.59)	20 (71.43)	
Physical activity *n* (%)			
Yes	13 (48.15)	5 (17.86)	$\chi^2 = 7.01$, $p < .05$
No	14 (51.85)	23 (82.14)	
BMI *M (SD)*	21.50 (2.55)	21.57 (2.92)	$t_{(47.89)} = -.09$, *ns.*
Caffeine consumption *n* (%)			
Yes	12 (44.44)	12 (42.86)	$\chi^2 = .01$, *ns.*
No	15 (55.56)	16 (57.14)	
Use of contraceptives *n* (%)	6 (22.22)	4 (14.29)	$\chi^2 = .58$, *ns*
PSQI *M (SD)*	4.52 (1.87)	6.86 (3.00)	$t_{(53)} = -.3{,}45$, $p < .01$
Amount of sleep *M (SD)*	7.39 (1.38)	6.84 (1.31)	$t_{(53)} = -.31$, *ns.*
Global Vigor *M (SD)*			
Morning	64.36 (19.61)	46.05 (20.05)	CT[a]: F(1,51) = 8.75, p < .01
Evening	67.52 (14.38)	56.79 (17.90)	TT[b]: F(1,51) = 2.00, ns.
Global Affect *M (SD)*			
Morning	71.20 (12.74)	64.89 (14.06)	CT: $F_{(1,51)} = 1.22$, *ns.*
Evening	71.35 (14.03)	69.61 (13.25)	TT: $F_{(1,51)} = .39$, *ns.*
SVF *M (SD)*			
Positive strategies	12.29 (2.65)	12.12 (2.76)	$t_{(53)} = .24$, *ns.*
Negative strategies	12.45 (3.98)	11.23 (3.81)	$t_{(53)} = 1.16$, *ns.*

[a] CT: main effect chronotype.
[b] TT: main effect testing time.

3.1.3. SVF

The likelihood of applying positive or negative coping strategies was equal in M- and E-types.

3.2. Physiological and subjective measures

Means and Standard Deviations of physiological and subjective measures are depicted in Table 2. To test for significant influences of trial, chronotype and testing time, we conducted a 2 (trial)×2 (chronotype)×2 (testing time) ANOVA. Dependent variables were HR (Fig. 1), SBP, DBP RMSSD (Fig. 2), LF/HF, and subjective stress ratings. Interactions of testing time×trial were significant for HR ($F_{(1,49)} = 6.28$, $p < .05$), SBP ($F_{(1,51)} = 8.07$, $p < .01$), RMSSD ($F_{(1,49)} = 8.70$, $p < .01$) and LF/HF ($F_{(1,49)} = 4.70$, $p < .05$), but not for subjective stress ratings ($F_{(1,51)} = 1.56$, *ns.*) and DBP ($F_{(1,51)} = 2.34$, *ns.*); all other interactions were non-significant (all $F_{(df)} < 2.20$, *ns.*). Post hoc pair-wise comparisons revealed that at baseline, all measures were independent of testing time (all $p > .10$, *ns.*). Under stress, differences between the two testing times were marginally significant (all $0.5 < p > .10$) with HR, SBP and LF/HF being higher and RMSSD being lower in the evening than in the morning.

We found a main effect for trial for all physiological measures: HR ($F_{(1,49)} = 225.98$, $p < .01$), SBP ($F_{(1,51)} = 369.29$, $p < .01$), DBP ($F_{(1,51)} = 423.76$, $p < .01$), RMSSD ($F_{(1,49)} = 113.16$, $p < .01$), LF/HF ($F_{(1,49)} = 104.18$, $p < .01$), and subjective stress ratings ($F_{(1,51)} = 459.30$, $p < .01$). During mental arithmetic, HR, SBP, DBP, LF/HF and subjective ratings were significantly higher than at baseline while RMSSD was significantly lower, indicating appropriate sympathetic activation during stress.

Chronotype had a significant main effect on HR ($F_{(1,49)} = 7.82$, $p < .00$), SBP ($F_{(1,51)} = 4.91$, $p < .05$), RMSSD ($F_{(1,49)} = 7.23$, $p = .01$) and on subjective stress ratings ($F_{(1,51)} = 4.81$, $p < .05$), but not on LF/HF ($F_{(1,49)} = 1.11$, *ns.*) and DBP ($F_{(1,51)} = .73$, *ns.*). M-types had significantly higher RMSSD, whereas HR, SBP and subjective stress ratings were lower than in E-types. Testing time had no significant effect on any of the measures.

Table 2
Means and standard deviation of physiological and subjective measures.

	Baseline				Stress			
	Morning		Evening		Morning		Evening	
	M-types	E-types	M-types	E-types	M-types	E-types	M-types	E-types
HR	70.90 (12.02)	80.40 (11.73)	73.19 (7.77)	80.04 (6.16)	92.59 (17.67)	104.53 (15.21)	102.85 (16.42)	110.99 (15.80)
SBP	99.31 (7.19)	103.79 (9.69)	97.92 (6.91)	101.83 (7.03)	119.48 (10.30)	126.21 (15.00)	125.95 (10.30)	131.02 (8.65)
DBP	69.17 (4.96)	71.79 (6.90)	68.95 (6.17)	70.31 (8.33)	84.29 (6.47)	86.52 (10.59)	86.69 (8.34)	87.21 (9.55)
RMSSD (ln)	3.74 (.49)	3.42 (.52)	3.78 (.52)	3.49 (.41)	3.33 (.57)	2.89 (.55)	3.00 (.61)	2.53 (.72)
LF/HF (ln)	−.05 (.98)	−.03 (1.02)	−.36 (1.11)	.13 (.82)	.88 (.60)	1.27(.40)	.78 (.65)	.67 (.39)
Subj. rating	1.93 (1.54)	2.57 (1.87)	2.15 (1.77)	2.57 (1.51)	7.93 (1.27)	8.21 (1.97)	7.62 (1.98)	9.36 (1.65)

3.3. Change scores

For all dependent variables, stress reactivity was defined as the difference between baseline and mental arithmetic. A higher change score reflects a greater change and thus higher reactivity to mental stress. 2 (chronotype) × 2 (testing time) ANOVAs were conducted for all dependent variables (see Table 3 for means, standard deviations and test statistic of change scores). Change scores were influenced by testing time such that HR ($F_{(1,49)}=3.45$, $p=.07$), and SBP ($F_{(1,51)}=10.10$, $p<.01$) rose to a greater extent when stress was induced in the evening as compared to the morning. At the same time RMSSD ($F_{(1,49)}=5.78$, $p<.05$) decreased more strongly in the evening. Neither M/E nor the interaction of M/E × testing time was significant for any of the change score variables (all $F_{(df)}<2.00$, *ns.*).

4. Discussion

The results of this study confirmed our first hypothesis about differences in cardiovascular resting levels between morning- and evening-types for HR, SBP, and HRV. Morning-types had significantly lower HR and SBP and significantly higher HRV, as indexed by RMSSD. In addition, we were able to replicate previous findings of other studies, such that M-types showed better sleep quality [55], smoked less frequently [11] and were more physically active [54] as compared to E-types.

Significant main effects of trial for all dependent variables demonstrate successful stress induction. Absolute levels of HR, SBP, DBP, subjective stress ratings and sympathovagal balance were significantly higher during stress than at baseline, whereas the reverse was true for RMSSD. Interactions of trial × testing time were significant for absolute levels of HR, SBP, RMSSD, and LF/HF and were mainly due to a stronger effect of stress induced in the evening as compared to the morning. Baseline corrected change scores of stress reactivity confirmed these results as HR and SBP rose to a greater extent when stress was induced in the evening as compared to the morning. Again, the reverse was true for RMSSD. These findings suggest that stress induced in the evening had a significantly higher impact on cardiovascular responses than stress induced in the morning independent of chronotype. Thus, we could not confirm our second hypothesis of higher stress reactivity in E-types. Subjective stress ratings did not reflect physiological differences between testing times. The main effect of testing time for cardiovascular activity under stress is in contrast with the results reported by Nebel and colleagues [35] and Willis and colleagues [36], who did not find that stress in the evening had stronger effects than stress in the morning. Though other explanations, e.g. characteristics of the sample, have to be taken into account, the lack of this effect might be due to their earlier testing time (12:30–2 p.m. vs. 4–7 p.m. in our study). Taking the findings together, we conclude that M/E is related to different levels of HR, SBP, and HRV at rest and under stress. E-types present with lower absolute levels of HRV and higher levels of HR and SBP than M-types. Both M- and E-types, however, responded with higher reactivity when tested in the evening as compared to the morning.

In contrast to our third hypothesis we failed to replicate the interaction of M/E and time of day found by Nebel and colleagues [35]. While Willis and colleagues [36] reported this so called "synchronicity effect" for E-types in their sample, no interaction was significant in our study. An obvious difference between the three experiments – and therefore a possible influencing factor – is the sex of the sample. In contrast to Nebel and colleagues [35], who tested male subjects only, Willis and colleagues [36] had a mixed sample, while ours was completely female. Furthermore, we realized a between-subjects design to avoid habituation to the stress induction and thereby, focused on inter-individual differences between chronotypes. This design was not suitable to account for intra-individual fluctuations during the day. Two other characteristics of our study sample imply limits of our study: We applied the 20-percentile criterion to recruit subjects from our screening sample and thereby, extended the original MEQ-categories for moderate E- and M-types. We accepted the risk of recruiting intermediate chronotypes and attenuating the group comparison. Another possible limitation regarding sample selection is that the majority of our sample (74.6%) were students of study subjects with restricted admission, i.e. to be enrolled requires excellent academic grades. As motivation regulation can be used to predict classroom performance [56], students of these challenging subjects might have learned to overcome their diurnal ups and downs to perform well.

Several findings about M/E and vulnerability to stress have shown a strong tendency for eveningness to relate to less efficient self-regulation. E-types seem to be less emotionally stable than M-types [14,15], less satisfied with life [17], and show a higher prevalence of psychosomatic symptoms [16]. Furthermore, eveningness has been found to correlate positively with depression [22], eating disorders [24,25], and problems in coping with environmental and social demands [16]. Therefore, we assumed a lack of self-regulation in E-types as compared to M-types. The central autonomic network connects self-regulatory strength and HRV. Because of this relationship

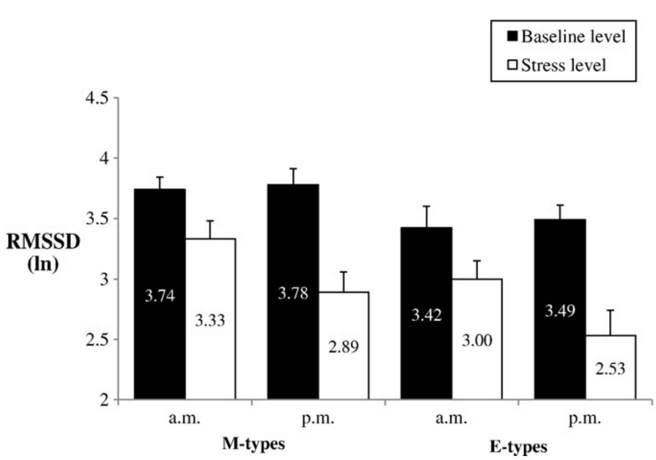

Fig. 2. RMSSD (ln) at baseline and stress level. Error bars indicate standard error.

Table 3
Means, standard deviations and test statistic of change scores.

	M-types		E-types		
M (SD)	Morning	Evening	Morning	Evening	Main effect TT
HR	21.69 (13.16)	24.14 (13.50)	29.67 (12.61)	30.83 (17.97)	$F_{(1,49)} = 3.45$, $p = .07$
SBP	20.17 (8.71)	22.43 (10.73)	28.03 (7.55)	29.19 (6.45)	$F_{(1,51)} = 10.10$, $p < .01$
DBP	19.12 (7.25)	19.10 (4.12)	15.88 (7.47)	19.52 (4.81)	$F_{(1,51)} = 1.20$, *ns.*
RMSSD (ln)	−.40 (.60)	−.53 (.62)	−.78 (.49)	−.98 (.78)	$F_{(1,49)} = 5.78$, $p < .05$
LF/HF (ln)	1.21 (.84)	1.35 (.97)	1.13 (.93)	.63 (.85)	$F_{(1,49)} = 1.12$, $p > .10$, *ns.*
Subj. rating	6.00 (1.84)	5.64 (2.82)	5.46 (2.82)	6.79 (1.97)	$F_{(1,51)} = .13$, $p > .10$, *ns.*

of prefrontal functions and HRV, reduced HRV in E-types might serve as a correlate for their increased impulsivity and decreased emotional stability as compared to M-types.

Nevertheless, it cannot be ruled out that sympathetic activation might be overestimated in stress trials like mental arithmetic that require speaking, due to more irregular breathing patterns [57]. This could explain why our findings were limited to RMSSD and not visible in sympathovagal balance. The LF/HF ratio might have been skewed by sympathetic dominance due to talking. However, it has been shown that the increase in HR due to the stressing effect of a mental arithmetic task outweighed the influence of speech [58,59]. No such findings exist for any parameters of HRV. Therefore, we recommend controlling for this aspect by using a non-verbal task or recording breathing rate in future studies. We further propose the investigation of a mixed sample and the inclusion of different age groups and educational levels e.g., by selecting subjects from a more heterogeneous population and analyzing subgroups of different sex, age and education.

Opposed to these caveats, the present study has several advantages. Firstly, as M/E has been shown to be a continuum [5], we consider an extreme-group comparison more appropriate than a median split as performed by Nebel and colleagues [35]. Secondly, we eliminated the skewing influence of sex on M/E that was found in our screening and on cardiovascular activity found by Willis and colleagues [36]. Thirdly, we chose a later time window (4–7 p.m.), which is more suitable to reflect peaks in performance and activity in E-types [37,38]. Fourthly, following Willis and colleagues [36], we used a mental stressor as this has proven more appropriate than physical stress [35]. Finally, group differences cannot be attributed to age, BMI, caffeine consumption, intake of medication or the amount of sleep the night before testing, because these variables did not vary significantly between chronotypes in our sample. We controlled statistically for group differences in the frequency of smoking or engaging in regular exercise physical activity.

Our results on HR, BP and HRV provide a physiological approach to explain the relationship between M/E and susceptibility to stress. Instead of an interaction of M/E and testing time, we found a general effect of chronotype on cardiovascular stress response. Therefore, our findings indicate that M/E is not only reflected in circadian variations in experiencing stress, but that M/E may account for profound differences in stress responses irrespective of time of day.

5. Conclusion

Eveningness is known to be associated with higher emotional instability, less effective handling of stress and increased vulnerability for mental and psychosomatic distress than morningness. The present findings demonstrate that E-types have lower HRV at rest and during stress, which can be considered a physiological correlate of behavioral and emotional difficulties. Regarding prevention of and intervention when mental or physical difficulties are encountered, particularly in E-types, measures of raising HRV such as HRV-Biofeedback might prove effective.

Conflict of interests

Neither author has any conflicts of interest.

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