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Chronotype and sleep duration: The influence of season of assessment

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Little is known about human entrainment under natural conditions, partly due to the complexity of human behavior, torn between biological and social time and influenced by zeitgebers (light–dark cycles) that are progressively "polluted" (and thereby weakened) by artificial light. In addition, data about seasonal variations in sleep parameters are scarce. We, therefore, investigated seasonal variation in cross-sectional assessments of sleep/wake times of 9765 subjects from four European populations (EGCUT = Estonian Genome Centre, University of Tartu in Estonia; KORA = Cooperative Health Research in the Region of Augsburg in Germany; KORCULA = The Korcula study in Croatia; and ORCADES = The Orkney Complex Disease Study in Scotland). We identified time-of-year dependencies for the distribution of chronotype (phase of entrainment assessed as the mid-sleep time point on free days adjusted for sleep deficit of workdays) in cohorts from Estonia (EGCUT) and Germany (KORA). Our results indicate that season (defined as daylight saving time – DST and standard zonetime periods – SZT) specifications of photoperiod influence the distribution of chronotype (adjusted for age and sex). Second, in the largest investigated sample, from Estonia (EGCUT; N = 5878), we could detect that seasonal variation in weekly average sleep duration was dependent on individual chronotype. Later chronotypes in this cohort showed significant variation in their average sleep duration across the year, especially during DST (1 h advance in social time from the end of March to end of October), while earlier chronotypes did not. Later chronotypes not only slept less during the DST period but the average chronotype of the population assessed during this period was earlier than during the SZT (local time for a respective time zone) period. More in detail, hierarchical multiple regression analyses showed that, beyond season of assessment (DST or SZT), social jetlag (SJl; the discrepancy between the mid sleep on free and work days – which varied with age and sex) contributed to a greater extent to the variation in sleep duration than chronotype (after taking into account factors that are known to influence sleep duration, i.e. age, sex and body mass index). Variation in chronotype was also dependent on age, sex, season of assessment and SJl (which is highly correlated with chronotype – SJl was larger among later chronotypes). In summary, subjective assessments of sleep/wake times are very reliable to assess internal time and sleep duration (e.g. reproducing sleep duration and timing tendencies related to age and sex across the investigated populations), but season of assessment should be regarded as a potential confounder. We identified in this study photoperiod (seasonal adaptation) and SJl as two main factors influencing seasonal variation in chronotype and sleep duration. In conclusion, season of assessment, sex and age have an effect on epidemiological variation in sleep duration, chronotype and SJl, and should be included in studies investigating associations between these phenotypes and health parameters, and on the development of optimal prevention strategies.

Keywords: Chronotype, DST, photoperiod, seasonality, sleep duration, social jetlag

INTRODUCTION

The current theories about the functionality of the human circadian system were investigated in constant routine or forced desynchrony protocols in isolation facilities (for review, see Foster & Roenneberg, 2008).

Changes in photoperiod are known to influence the sleep/wake behavior in humans (Chang et al., 2011; Danilenko et al., 2000) and in model organisms (i.e. clock genes expression; Ciarleglio et al., 2011; Wright et al., 2005). However, little is known about human

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entrainment under natural conditions (Arendt, 2012; Borisenkov, 2011; Emens et al., 2009), partly due to the complexity of human behavior, torn between biological and social time and influenced by zeitgebers (light–dark cycles) that are progressively ''polluted'' (and thereby weakened) by artificial light and less natural light exposure during the 24-h day.

Seasonal rhythms in humans were shown for body mass index (BMI), circadian markers (melatonin, rectal temperature and sleep–wakefulness) and rates of conception, mortality and suicide (for review, see Foster & Roenneberg, 2008; Honma et al., 1992; Roenneberg & Aschoff, 1990a, b). Seasonal adaptation, in central Europeans, was shown to be disrupted during the annual transition to daylight saving time (DST) (Kantermann et al., 2007) – this one-hour advance in social time increases the discrepancy between social and circadian timing (social jetlag (SJl), assessed by the difference between mid-sleep on workdays and free days; Roenneberg et al., 2007a), leading to sleep deprivation (Kantermann et al., 2007). Both SJl and short sleep duration are associated with obesity and the manifestation of depressive symptoms (Levandovski et al., 2011; Roenneberg et al., 2012), phenotypes that *per se* show circannual variation.

Studying populations from Estonia (EGCUT), Scotland (ORCADES), Germany (KORA Augsburg) and Croatia (KORCULA), we aimed to systematically identify seasonal (time of the year based on DST and standard zonetime (SZT) periods) dependencies in sleep patterns, based on cross-sectional data collected with the Munich Chronotype Questionnaire (MCTQ).

METHODS

Study cohorts

Data from 9765 phenotyped participants (all of European ancestry) from the cohorts EGCUT = Estonian Genome Centre, University of Tartu in Estonia, KORA F4 = Cooperative Health Research in the Region of Augsburg in Germany (a follow up of KORA S4; Wichmann et al., 2005), KORCULA = The Korcula study in Croatia and ORCADES = The Orkney Complex Disease Study in Scotland were included in these analyses. A full description of the participating cohorts has been published recently (Allebrandt et al., 2013). To identify possible confounders that might have influenced our earlier genetic association analyses (Allebrandt et al., 2013), we evaluated to which extent both season of assessment (DST or SZT = standard zone time) and chronotype can explain variations in sleep duration in our study cohorts. We collected consent for unrestricted use of the phenotype data for all cohorts described in this manuscript. Figure 1 provides sample characteristics and information about the cohorts studied here. Data collection with the MCTQ (Kantermann et al., 2007) started in 2007 and ended in 2009. EGCUT data were analysed for three

subpopulations, a discovery sample and two replication samples, used in our earlier genome-wide association studies (GWAS; for details, see Allebrandt et al., 2013). The KORA sample included in this study was twice as large as the original sample included in the GWAS studies. Informed consents were obtained from all participants, and the respective local ethics committees approved the study designs, meeting the ethical standards of this journal (Portaluppi et al., 2008).

Phenotyping

Sleep times were assessed with the MCTQ, which asks a series of simple questions regarding sleep timing on workdays (mostly depending on work times or timing of social activities) and on free days (associated with the individual's endogenous circadian rhythm). Chronotype was calculated using the mid-point of sleep on free days (days without social constraints, mid-sleep on free days (MSF); see also details of the phenotyping quality control below) and adjusted for sleep deficit on workdays (MSFsc; Roenneberg et al., 2007a). SJl was calculated as the difference between mid-sleep on workdays and free days. Regarding phenotyping quality control, inclusion criteria were as follows: (1) no use of an alarm clock on free days; (2) no shift-work during the last three months; (3) no use of sleep medication (benzodiazepines and other pharmacological agents that influence sleep; see Supplemental Material of Allebrandt et al., 2013); and (4) sleep duration between 3 and 12.5 h. Datasets were also cleaned to exclude replies with missing or inconsistent data (e.g. day sleep or extreme sleep latency).

Statistical analysis

We used non-parametric statistics to not violate assumptions of normality in the subgroup analyses. Mann–Whitney U tests were used for comparing distributions of two quantitative variables and Kruskal–Wallis tests for comparisons of three or more of these variables. The datasets were analysed for the earlier versus later half of the chronotype distribution (based on the MSFsc normalised for age and sex – MSF_{sasc}; Kantermann et al., 2007) separately for the DST period and SZT (Tables 1 and 2). To verify the distribution of mean average sleep duration and MSF_{sc} by age and sex, five age groups (see legend of Figure 1) were defined based on earlier studies (Allebrandt et al., 2010; Levandovski et al., 2011) and on the current sample sizes (see Figure 1). To identify variation in the weekly average sleep duration (adjusted for age and sex; see Allebrandt et al., 2010) and chronotype distributions across 12 months, we compared these by Kruskal–Wallis test for both subgroups of individuals belonging to the early or late half of the chronotype distribution in the large Estonian cohort (EGCUT replication 2). Multiple regression analysis was only conducted with this largest sample, which was powered for this analysis, having hundreds of assessments in every single month within a year, using average
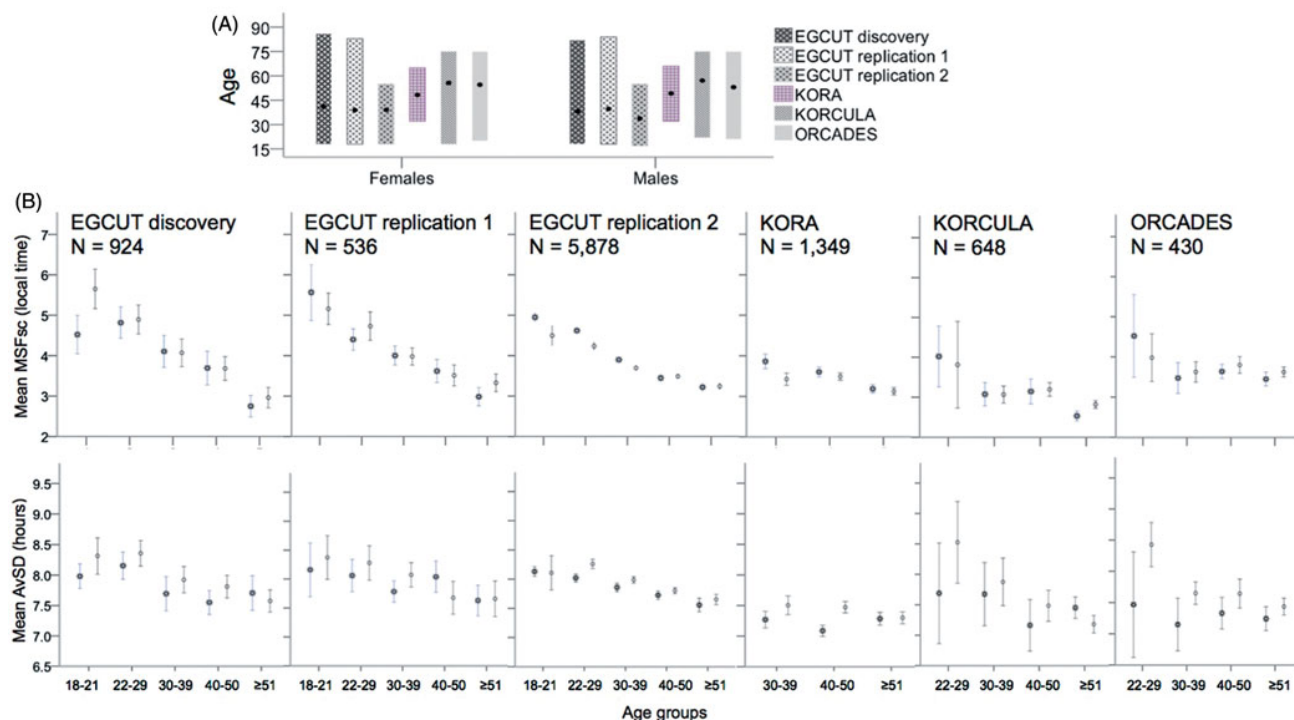
FIGURE 1. Study sample characteristics. (A) Mean, minimum and maximum age per sex are plotted for each of the participating cohorts (EGCUT, Estonia; KORA, Augsburg in Germany; KORCULA, the Korcula Island in Croatia; and ORCADES, the Orkney Islands in the United Kingdom). (B) Sleep duration and chronotype (MSF_{sc}) vary with age and sex. The mean for weekly averaged sleep duration shortens steadily, and the mean chronotype was continuously earlier in both sexes, for subjects at the end of adolescence to subjects at the age of menopause. On an average, women (light grey) sleep longer than men (dark grey). The age-dependency of chronotype (upper panel) and averaged weekly sleep duration (bottom panel) are plotted separately for women and men from five age groups: 18–21 years; 22–29 years; 30–39 years; 40–50 years; and ≥51 years. AvSD = average sleep duration; MSF_{sc} = mid-sleep on free days adjusted for sleep deficit on workdays. Vertical lines represent 95% confidence intervals.

TABLE 1. Independent Sample Mann–Whitney U test for MSF_{sasc} and mean weekly averaged SD_{asc} grouping by season.

Cohorts	Latitude	Settlements inhabitants	N	DST/SZT (%)	MSF_{sasc} Mean ± SEM (DST/SZT)	SD_{asc} Mean ± SEM (DST/SZT)
EGCUT discovery	57° N to 59° N	45%5 50 000[a]	924	43/57	4.22 ± 0.10/4.28 ± 0.07	7.99 ± 0.06/8.08 ± 0.04
EGCUT replication 1	57° N to 59° N	44%5 50 000[b]	536	68/32	4.22 ± 0.05/4.32 ± 0.09*	7.89 ± 0.05/7.89 ± 0.09
EGCUT replication 2	57° N to 59° N	73%5 50 000[c]	5878	55/45	4.06 ± 0.02/4.12 ± 0.02**	8.08 ± 0.02/8.12 ± 0.02*
KORA	48° N	& 300 000	1349	51/49	4.04 ± 0.03/4.16 ± 0.03*	7.67 ± 0.03/7.65 ± 0.03
KORCULA	42° N	& 20 000	648	52/48	3.75 ± 0.04/3.66 ± 0.05	7.45 ± 0.06/7.53 ± 0.08
ORCADES	59° N	& 20 000	430	49/51	4.42 ± 0.06/4.40 ± 0.06	7.51 ± 0.06/7.61 ± 0.06
Total			9765			

MSF_{sasc} = mid-sleep on free days adjusted for sleep deficit on workdays, normalised for age and sex (chronotype); SD_{asc} = sleep duration normalised for age and sex; SEM = standard error of the mean; DST = daylight saving time; SZT = standard zonetime. Cohorts and their respective populations: EGCUT, Estonia; KORA, Augsburg in Germany; KORCULA, the Korcula Island in Croatia; and ORCADES, the Orkney Islands in the United Kingdom. Percent of inhabitants per town = [a]23%4 100 000; 32% unknown; [b]54%4 100 000; 3% unknown; [c]24%4 100 000; 3% unknown. Levels of significance = p5 0.05*, and p5 0.01**.

sleep duration or MSF_{sc} as dependent variables (for independent variables, see Tables 3 and 4). MSF_{sc}, SJl and BMI were $\log^{10}$ transformed (+ constant, i.e. +2 and +4 for MSF_{sc} and SJl, respectively, before log transformation) for parametric statistics. Normality was confirmed by visual inspection of Q–Q plots (the large sample size makes the use of normality tests unreliable; Field, 2009). For the linear regression models, the indices of co-linearity were within the accepted range (tolerance5 1; for the variance inflation factor range,

see Tables 3 and 4). Analyses were performed with SPSS version 19 (SPSS Inc., Chicago, IL) for Macintosh.

RESULTS

Mean weekly average sleep duration decreased steadily, and mean chronotype was continuously earlier in both sexes between the age of 20 (around the end of adolescence; only available for the EGCUT cohorts from Estonia) and 55 years (around menopause; Figure 1).

TABLE 2. Independent sample Mann–Whitney U test for social jetlag grouping by chronotype.

Cohort	Early/late (N)	Total (N)	Mean rank (SJl earlier/later chronotypes)	Mean ± SEM (SJl earlier/later chronotypes)	Asymp. Sig.
			Assessments during DST period		
EGCUT discovery	201/192	393	137/259	0.73 ± 0.05/2.02 ± 0.094	1.21^{-26}
EGCUT replication 1	188/177	365	155/213	0.84 ± 0.052/1.39 ± 0.076	1.17^{-7}
EGCUT replication 2	1656/1577	3233	1294/1956	1.03 ± 0.018/1.65 ± 0.023	2.13^{-90}
KORA	363/331	694	284/417	0.83 ± 0.038/1.29 ± 0.039	3.40^{-18}
KORCULA	156/179	335	150/184	0.15 ± 0.031/0.34 ± 0.039	9.45^{-5}
ORCADES	99/113	212	105/108	0.35 ± 0.047/0.39 ± 0.046	n.s.
Total		5232			
			Assessments during standard zonetime		
EGCUT discovery	260/271	531	215/315	0.78 ± 0.046/1.52 ± 0.075	4.94^{-14}
EGCUT replication 1	80/91	171	67/103	0.76 ± 0.081/1.52 ± 0.119	2.75^{-6}
EGCUT replication 2	1283/1362	2645	1055/1576	1.11 ± 0.022/1.75 ± 0.027	4.89^{-69}
KORA	312/343	655	268/383	0.86 ± 0.038/1.35 ± 0.048	8.04^{-15}
KORCULA	166/147	313	138/179	0.18 ± 0.030/0.47 ± 0.053	3.50^{-6}
ORCADES	116/102	218	91/131	0.33 ± 0.039/0.64 ± 0.055	2.05^{-6}
Total		4533			

DST = daylight saving time period; SJl = social jetlag.
Later chronotypes had on an average higher amounts of social jetlag (mean ranks in bold) than earlier chronotypes.

TABLE 3. Hierarchical multiple regression analysis with mean sleep duration as dependent variable.

Model		Unstandardised coefficients		Standardised coefficients	95.0% confidence interval for B	
		B	Standard error	Beta	Lower bound	Upper bound
Step 1	(Constant)	8.504	0.042		8.421	8.587
	Age	−0.016	0.001	−0.188***	−0.019	−0.014
Step 2	(Constant)	8.361	0.050		8.263	8.459
	Age	−0.018	0.001	−0.206***	−0.020	−0.016
	Sex	0.132	0.025	0.071***	0.084	0.181
Step 3	(Constant)	8.769	0.224		8.330	9.208
	Age	−0.017	0.001	−0.197***	−0.020	−0.015
	Sex	0.125	0.025	0.067***	0.076	0.174
	Log BMI	−0.304	0.162	−0.025	−0.622	0.014
Step 4	(Constant)	9.399	0.268		8.873	9.924
	Age	−0.019	0.001	−0.220***	−0.022	−0.017
	Sex	0.137	0.025	0.073***	0.088	0.186
	Log BMI	−0.320	0.162	−0.027*	−0.638	−0.002
	SJl (+4) log-transformed	−0.763	0.179	−0.059***	−1.115	−0.411
Step 5	(Constant)	9.360	0.269		8.833	9.887
	Age	−0.019	0.001	−0.221***	−0.022	−0.017
	Sex	0.138	0.025	0.074***	0.089	0.187
	BMI log transformed	−0.330	0.162	−0.028*	−0.648	−0.012
	SJl (+4) log-transformed	−0.789	0.180	−0.061***	−1.142	−0.437
	Season code	0.050	0.024	0.027*	0.003	0.097
Step 6	(Constant)	9.541	0.289			
	Age	−0.020	0.001	−0.228***	8.975	1.107
	Sex	0.132	0.025	0.071***	−0.023	−0.017
	Log BMI	−0.341	0.162	−0.029*	0.082	0.181
	SJl (+4) log-transformed	−0.627	0.203	−0.048**	−0.659	−0.023
	Season code	0.050	0.024	0.027*	−1.026	−0.228
	MSF$_{sc}$ (+2) log-transformed	−0.328	0.192	−0.028	0.003	0.097

Summary of the 6-step hierarchical multiple regression model (enter method). $r^2 = 0.035$ ($F_{(1,5868)} = 215.89$), $p5$ 0.000) for Step 1, $r^2_{change} = 0.05$ ($F_{(1,5867)} = 28.91$, $p5$ 0.000) for Step 2, $r^2_{change} = 0.01$ ($F_{(1,5866)} = 3.51$, $p = 0.06$) for Step 3, $r^2_{change} = 0.03$ ($F_{(1,5865)} = 18.08$, $p5$ 0.000) for Step 4, $r^2_{change} = 0.01$ ($F_{(1,5864)} = 4.42$, $p = 0.036$) for Step 5, and $r^2_{change} = 0.00$ ($F_{(1,5863)} = 2.92$, $p = 0.088$) for Step 6. Exchanging the position of social jetlag with MSF$_{sc}$ in the hierarchical model did not improve the model fit – steps 4, 5 and 6 become respectively, $r^2_{change} = 0.02$ ($F_{(1,5865)} = 12.25$, $p = 0.000$) for Step 4, and $r^2_{change} = 0.02$ ($F_{(1,5864)} = 3.67$, $p = 0.055$ for Step 5, and $r^2_{change} = 0.02$ ($F_{(1,5863)} = 9.49$, $p = 0.002$) for Step 6. BMI = body mass index; SJl = social jetlag; MSF$_{sc}$ = mid-sleep on free days adjusted for sleep deficit on workdays (chronotype). VIF values ranged from 1 to 1.66 in step 6. B: unstandardised regression coefficients; SE: standard error; b: standardised regression coefficients; CI: confidence intervals (lower to upper bound). Levels of significance = $p5$ 0.05*; $p5$ 0.01** and $p5$ 0.001***.

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TABLE 4. Hierarchical multiple regression analysis with MSF_{sc} (chronotype) as dependent variable.

Model		Unstandardised coefficients		Standardised coefficients	95.0% confidence interval for B	
		B	Standard error	Beta	Lower bound	Upper bound
1	(Constant)	0.891	0.003		0.884	0.897
	Age	−0.004	0.000	−0.472***	−0.004	−0.003
2	(Constant)	0.902	0.004		0.895	0.910
	Age	−0.003	0.000	−0.456***	−0.004	−0.003
	Sex	−0.011	0.002	−0.067***	−0.014	−0.007
3	(Constant)	0.896	0.005		0.887	0.905
	Age	−0.003	0.000	−0.456***	−0.004	−0.003
	Sex	−0.011	0.002	−0.067***	−0.014	−0.007
	Season code	0.004	0.002	0.028*	0.001	0.008
4	(Constant)	0.508	0.010		0.487	0.528
	Age	−0.002	0.000	−0.282***	−0.002	−0.002
	Sex	−0.019	0.002	−0.116***	−0.022	−0.015
	Season code	−9.760E-5	0.002	−0.001	−0.003	0.003
	SJl (+ 4) log-transformed	0.496	0.012	0.445***	0.472	0.520

Summary of a four-step hierarchical multiple regression model (enter method). $r^2 = 0.223$ ($F_{(1,5876)} = 1688.62$, $p5$ 0.000) for Step 1, $r^2_{change} = 0.04$ ($F_{(1,5875)} = 32.26$, $p5$ 0.000) for Step 2, $r^2_{change} = 0.01$ ($F_{(1,5874)} = 5.78$, $p = 0.016$) for Step 3, $r^2_{change} = 0.169$ ($F_{(1,5873)} = 1642.95$, $p5$ 0.000) for Step 4. VIF values ranged from 1 to 1.25. B: unstandardised regression coefficients; SE: standard error; b: standardised regression coefficients; SJl = social jetlag; CI: confidence intervals (lower to upper bound). Levels of significance = $p5$ 0.05*; $p5$ 0.01** and $p5$ 0.001***.

Note that KORA Augsburg in Germany, KORCULA in Croatia and ORCADES in the Orkney Islands, UK, had mostly elderly subjects). Subsequent analyses, therefore, were adjusted for both age and sex. Figure 1 shows the means of MSF_{sc} and weekly average sleep duration across age groups and for females and males separately.

The percentage of subjects assessed during DST and SZT periods was comparable for three of the four cohorts (Table 1). An overrepresentation of assessments during the DST period was only observed for the EGCUT replication 1 subsample from Estonia, and thereby should not have influenced the results obtained with other cohorts. Total sample size of cohorts was variable, and we emphasise that significant results for the statistics described below were obtained for the largest samples (except for EGCUT replication 1, a relatively small sample, but with 68% of assessments conducted during DST). Thereby, the described effects may be also dependent of sample sizes.

Seasonal variation on chronotype, sleep duration distribution and SJl

Mean chronotype (adjusted for age and sex – MSF_{sasc}; Roenneberg et al., 2007b) tended to be later during SZT compared to DST. This difference was only found significant in three of the six population samples (the EGCUT replication subsamples 1 and 2 and KORA cohort; Table 1). In these cohorts, later chronotypes showed higher amounts of SJl than earlier chronotypes ($p5$ 0.0001, Mann–Whitney U test). In addition, these differences (mean ranks for SJl in Table 2) were exacerbated for data collected during DST (p values were smaller than for SZT period data comparisons; Table 2). In the Orkney Islands population (ORCADES in

Scotland), we neither observed seasonal differences in chronotype nor differences in SJl between earlier and later chronotypes during the DST period (Tables 1 and 2). However, differences in SJl were observed for the SZT assessments (Table 2). A borderline significant difference in chronotype, with an opposite tendency – later mean chronotype in summer – was only observed in the KORCULA Island cohort from Croatia (Table 1). The KORCULA population showed comparable differences in SJl between earlier and later chronotypes in both the DST and SZT period (p values for mean ranks; Table 2).

Analysing the monthly changes in sleep duration and chronotype in the largest investigated population from Estonia (EGCUT; Figure 2), we identified that later chronotypes (later half of the chronotype distribution; $MSF_{sasc} = 4.82 \pm 0.0002$) advanced their chronotype during those months in which the duration of daylight hours increased (≥ 12 h; DST period; Figure 2, right panel; comparing chronotype distributions during DST and SZT period by Mann–Whitney U test, independently for the earlier, $p = 0.67$, and later, $p5$ 0.05, halves of the total distribution). As subjects are forced to wake up one hour earlier during DST and are exposed to longer photoperiods (especially in northern regions), later chronotypes assessed during DST are slightly earlier than in the rest of the year and have more irregular sleep times from work to free days (represented here by SJl) to compensate sleep duration during DST. Supporting our hypothesis, sleep duration in earlier chronotypes in the largest EGCUT sample was similar throughout all 12 months of the year ($N = 2939$, $\chi^2_{(df=11)} = 16.828$, $p = 0.11$; Kruskal–Wallis test), while among later chronotypes there was a significant variation ($N = 2939$, $\chi^2_{(df=11)} = 21.008$, $p = 0.03$; Kruskal–Wallis test), especially
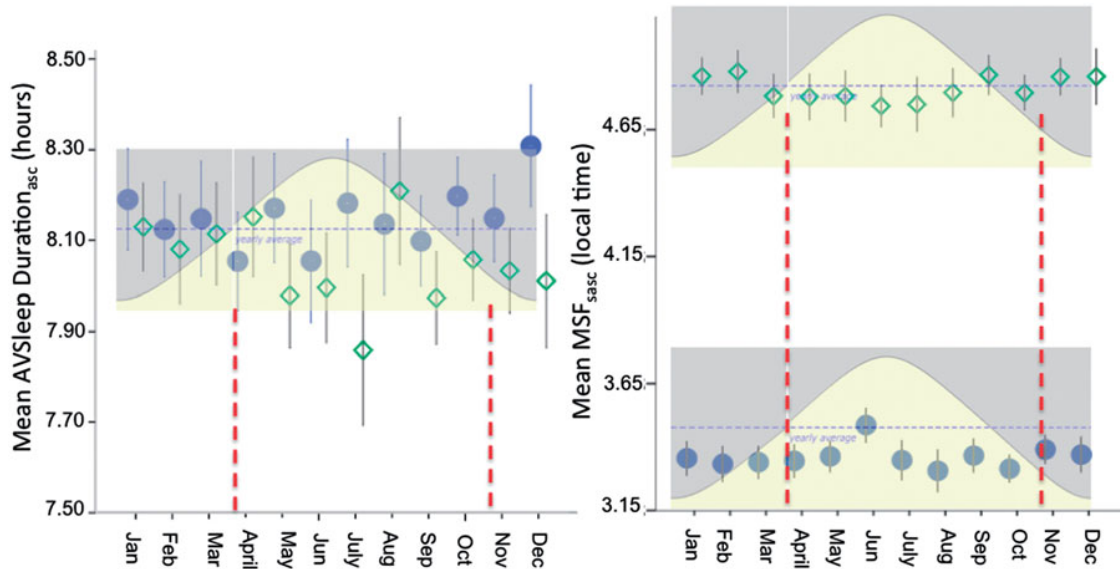
FIGURE 2. Mean weekly averaged sleep duration (adjusted for age and sex; left panel) and chronotype based on MSF (mid-sleep on free days adjusted for sleep deficit on workdays, age and sex; right panel) assessed across the year in the Estonian replication 2 sample. The sleep duration mean of earlier chronotypes (early half of the chronotype distribution; filled dots in the left panel) does not significantly vary across the year (with the longest mean in December; the darkest month of the year), while for late types (late half of the chronotype distribution; empty diamonds in the left panel) there is greater variation, especially during the DST period (dashed lines). After DST transition and with increasing photoperiod (over the yearly average hours in Estonia, with the longest day length in the 21st of June to 18 h and 20 min), sleep duration steadily decreases in later chronotypes until July. With increasing photoperiod, around DST transition in Spring to the end of summer, the mean chronotype of late chronotypes in Estonia (empty diamonds in the right panel) is relatively earlier than in periods with less daylight (from September to February), while the mean chronotype of earlier types (filled dots in the right panel) is particularly late in June (the month with the longest days). In August, with day length decreasing from 16 h and 30 min to 14 h and 7 min, later chronotypes recover sleep duration, which steadily decreases with photoperiod until December. From November until the end of March (standard zonetime), there was no significant variation in sleep duration among later chronotypes in this cohort. The numbers and percentages of assessments per month were as follows: January, 550 (9.4%); February, 524 (8.9%); March, 461 (7.8%); April, 450 (7.7%); May, 440 (7.5%); June, 387 (6.6%); July, 293 (4.9%); August 323 (5.5%); September 628 (10.7%); October 712 (12.1%); November 739 (12.6%) and December 371 (6.3%). Error bars represent 95% confidence interval. The overlaying graphic representing daylight hours across the year in Estonia was created with: http://astro.unl.edu/classaction/animations/coordsmotion/daylighthoursexplorer.html.

during DST (Figure 2). This variation was also found when mean weekly average sleep duration was compared between DST and SZT, independently for the earlier ($MSF_{sasc} = 3.36 \pm 0.0001$) and later half ($MSF_{sasc} = 4.82 \pm 0.0002$) of the chronotype distribution (Mann–Whitney U test, $p = 0.08$ and $p \leq 0.05$, respectively; Figure 2, left panel).

Therefore, assessments of internal time during the DST period can be assumed to be less reliable because of masking due to higher amounts of sleep deprivation. To elaborate why sleep duration was shorter during DST in this cohort, we investigated the relation between sleep duration, chronotype and SJl using a hierarchical multiple regression analyses. We found that both season and SJl influenced sleep duration (after entering those factors into the analyses that are known to influence sleep duration, i.e.: age, sex and BMI; Table 3). Although later chronotypes showed larger variation in sleep duration than earlier chronotypes, SJl had more weight in the regression model than chronotype (MSF_{sc}) as only SJl remained significant when both factors entered the analysis (Beta $= -0.048$, $p \leq 0.01$; Table 3). Chronotype (MSF_{sc}) and SJl were highly correlated (Pearson correlation, $r_{(5870)} = 0.55$, $p \leq 0.0001$). MSF_{sc} only contributed

to the variance in sleep duration when SJl was not entered in the analyses (Beta $= -0.052$, $p \leq 0.01$). Thus, the results above indicate that SJl had a larger effect than chronotype on the seasonal variation in sleep duration. Similarly, the variation in chronotype across the year was mostly dependent on age, sex, SJl and season of assessment (irrespective of the order SJl or season of assessment were entered into the hierarchic multiple regression model; Table 4).

Ancillary analyses

SJl was found to vary with age and sex (data from the largest EGCUT Estonian sample; Figure 3, Kruskal–Wallis test: for women, $N = 2966$, $\chi^2_{(df=4)} = 199.5$, $p \leq 0.0001$; and men: $N = 2912$, $\chi^2_{(df=4)} = 588.0$, $p \leq 0.0001$). Between 30 and 55 years of age, women had significantly more SJl than men (Figure 3, $p \leq 0.0001$, Mann–Whitney U test), but in both sexes, SJl was higher in adolescents and young adults than in adult life (Figure 3). Women were on an average earlier chronotypes than men in subgroups of the population with the same amounts of SJl (Figure 3). This finding was independent of age for most of the SJl categories (which did not all had a representative number of
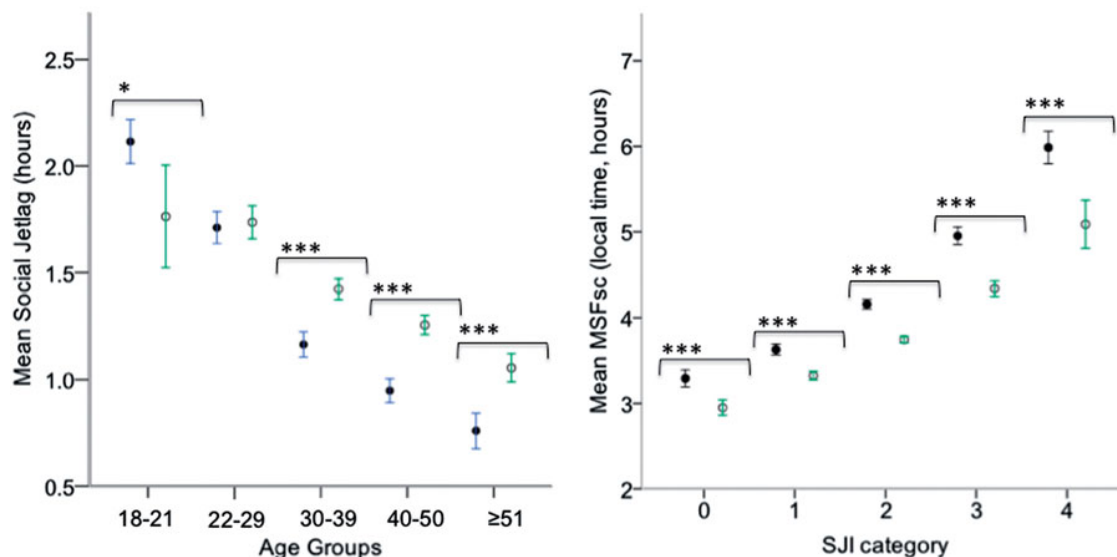
FIGURE 3. Social jetlag (SJl) varies with age and sex. From 18 to 21 years old (18–21 years: 63 women and 544 men), women (open circles) had on an average less social jetlag than men (filled circles) in the EGCUT replication 2 cohort (left panel). From this age group to the group of the elderly (age groups; 22–29 years: 516 women and 704 men; 30–39 years: 913 women and 694 men; 40–50 years: 1067 women and 698 men and ≥51 years of age: 407 women and 272 men), social jetlag decreased in both sexes. From 22 years old on, women continuously had significantly more social jetlag than men. Comparisons were conducted within SJl categories: 0 to 5 1 h (305 women and 489 men), 1 to 5 2 h (822 women and 803 men), 2 to 5 3 h (1331 women and 1007 men), 35 4 h (418 women and 429 men) and ≥4 h (90 women and 184 men), and significance levels are indicated in the graphic (left panel). For the same amount of social jetlag (categories 0 to above 4 h; right panel), women were in general earlier chronotypes than men. This was independent of season (data not shown), and some variation was observed in the MSF_{sc} distributions, of women and men in these SJl categories, for the different age groups (Supplementary Figure 1). Mann–Whitney U test, $p5$ 0.05*; $p5$ 0.01** and $p5$ 0.001***. Error bars 95% confidence interval.

men and women per each age group; Supplementary Figure 1). In addition, in the age category 18–21 years, there were almost nine times more men than women, which might have confound the results for this age category (Figures 1 and 3).

DISCUSSION

Influences of environmental time cues (such as time of year and amplitude of seasonal changes in day length) on human sleep behavior (as shown here and under controlled conditions; Chang et al., 2011; Danilenko et al., 2000), mental well being (for review, see Arendt, 2012; McClung, 2011; Sansone & Sansone, 2013) and energy metabolism (Patel, et al., 2012; Van Staveren et al., 1986) support a link between these systems. This study shows that season of assessment (DST and SZT period), beyond sex and age, influences sleep duration, chronotype and SJl, emphasising the relevance of these factors in studies investigating the associations of these phenotypes with adverse health outcomes.

Sleep duration, chronotype and SJl across different age groups
The variations in chronotype and weekly average sleep duration with age and sex that we observed are consistent with previous reports (Allebrandt et al., 2010; Roenneberg & Merrow, 2007). Sex differences in chronotype, however, were less evident than in earlier

studies, most likely because the sample sizes in this study were at least 10 times smaller than in those studies averaging data of groups of subjects within 4 (for the first bin) to 10 years, instead of averaging data for each year of age (Roenneberg et al., 2007a). The confounding influences of these systematic dependencies were dealt with through either normalisation procedures or using age and sex as covariates in the analyses (both methods lead to comparable results). Similar to chronotype, which advanced gradually from adolescence to late adulthood, the amount of SJl also decreased proportionally in the same age groups in our cohorts. When subjects enter adulthood, both social and genetic influences (modifications in gene expression) may contribute to more regular sleep/wake schedules, which would reduce SJl in both sexes. As women showed higher SJl than men, they might be at higher risk in developing adverse health symptoms associated with SJl, such as, for example, obesity (Roenneberg et al., 2012) and depressive symptoms (Levandovski et al., 2011).

Photoperiod length and phase of entrainment
Different aspects of light exposure play a role in circadian entrainment; day length (photoperiod) depends on time of year and the amplitude of its seasonal changes on latitude. Human entrainment is predominantly linked to dawn (Kantermann et al., 2007; Roenneberg et al., 2013). Therefore, an advance in phase of entrainment (considered in this study to be

the mid-sleep phase, chronotype), relative to the previous entrained phase (aftereffects; for review, see Johnson et al., 2003), can be expected during the seasonal transition towards longer photoperiod and vice versa (Borisenkov, 2011; Murray et al., 2003). This is supported by findings under controlled light paradigms, showing that the human circadian system adapts to prior photic history (Chang et al., 2011), and that perinatal photoperiod in mice determines the responsiveness of the biological clock to subsequent changes in photoperiod (Ciarleglio et al., 2011). Consistent with these findings, almost all northern populations had a clear tendency to be earlier chronotypes when photoperiods were longest, except The Orkney Island (ORCADES in Scotland) population. As this population is largely comprised of farmers, this could confound their sleep/wake times on weekends (not reflecting internal time, but work times) and thereby, no seasonal difference could be detected. As we are using cross-sectional data, we cannot identify how specific individuals would behave across seasons and clarify if, for instance, occupation would influence sleep/wake times on free days. The Korcula Island (KORCULA in Croatia) has a comparable population size with the Orkney Islands, but the economy in the former is rather based on tourism than on agriculture. Assessments in Estonia (EGCUT) were performed countrywide, hence covering urbanised and mixed population in terms of occupation, similar as for the KORA population in Augsburg, Germany. Populations in more urbanised areas might be less responsive to variations in dawn and dusk because of less overall exposure to environmental light (Roenneberg et al., 2007b). If seasonal adaptation then is delayed in these populations, the transition from SZT to DST period will be more significant for individuals with regular sleep times.

The Korcula Island in Croatia was the most southern of the investigated cohorts with the shortest photoperiod of 8.9 h (to a maximum of 12 h) during the SZT period, and the longest photoperiod of 15.1 h (from a minimum of 12 h) during the DST period. Winter days at that latitude are, therefore, longer than in the northern locations of the other cohorts included in our study. Therefore, it can be suggested that the seasonal transition at the latitude of Korcula Island influences internal time less. In addition, this might be one reason for the absence of a significant variation in chronotype between DST and SZT on that island. Notably, seasonal adaptation has only been studied little in humans under natural conditions, especially comparing populations living at different latitudes, leaving room for more future investigations. However, increasing light intensity (mean annual light intensities increase towards the Equator) is known to advance the phase of entrainment in most day-active animals (Aschoff's rule; for review, see Johnson et al., 2003). Synchronisation of individual circadian clocks by light has been consistently shown to be the strongest signal influencing sleep/wake times over social cues (Roenneberg et al., 2013). In this study, the variation in longitudinal location within investigated cohorts was rather low. Populations from the Orkney Islands (2.55° W; ORCADES) and Korcula Islands (16° to 17° E; KORCULA) were within one degree of longitude, while KORA population comes from a single town (Augsburg, 10° E in Germany). The EGCUT assessments were done all over Estonia, ranging from 23 to 26° of longitude east. Thereby, wake-up times are not expected to be influenced by longitude, as reported earlier for a German cohort (Roenneberg et al., 2007b).

It is important to note that we did not normalise the data that was collected under DST to SZT (1 h advance in social time during the summer months) because we cannot predict for how long subjects will be sleep deprived due to DST transition (or when seasonal adaptation took place). As seasonal adaptation can be expected to occur, individuals may adapt to DST transition at some time point during the DST period. That means that subjects in all cohorts would wake up earlier during DST (1 h advance in social time plus the differences we observed in this study). But, unfortunately, it was not possible to differentiate between effects related to DST and photoperiod (seasonal adaptation) in this study. Thus, chronotype and sleep duration assessments may be better representing biological sleep tendencies when assessed during the months with SZT (generally between October and March) than during DST. Ideally, associations with these phenotypes should be analysed using DST or month of assessment as co-variants. As human sleep/wake times track sun time under natural conditions, the phase advance during DST transition is artificial and therefore subjective assessments conducted during the DST period may not optimally represent internal time in subjects that also are more prone to sleep deficits. More studies are warranted to scrutinise our findings here.

Seasonal influence

This study shows a larger variation in sleep duration among later chronotypes assessed across the year in the Estonian cohort. This could be related to both seasonal adaptation, which is a continuous process, and DST transition, which challenges biological clock for an abrupt one-hour advance in time in spring. In earlier work from our group focusing on the DST transition (Kantermann et al., 2007), variation in mid-sleep was shown to start already about three months earlier than the DST transition to summer time following dawn and dusk. In assessments all over Germany ($N = 55\,000$), sleep duration was shorter during DST in comparison to the rest of the year. In this study, we show that only late chronotypes reported on average shorter sleep during DST (using the largest Estonian cohort, $N = 5878$), which also indicates sleep deficits in these later chronotypes. To investigate the influence of social times, we used

the difference in the mid-sleep time points on workdays and free days (SJl) to explain the variation in sleep duration. SJl had a larger effect on the variation of sleep duration than chronotype *per se*, in addition to season of assessment (DST or SZT). The strong correlation between chronotype and SJl is related to the greater difficulties later chronotypes have to adjust to early work schedules. The result is insufficient sleep during the workweek and especially during daylight-savings-time and longer photoperiods. Indeed, in both the DST and SZT assessments, we found higher amounts of SJl in later chronotypes in almost all cohorts (mean ranks marked in bold in Table 2). Only in the cohorts from the Orkney (Scotland) and Korcula Islands (Croatia), there were no or less differences in SJl between later and earlier chronotypes than in other cohorts (*p* values; Table 2). These cohorts did not show seasonal (DST opposed to SZT) epidemiological variation in sleep duration and chronotype. In summary, in this study, we show that both season of assessment and SJl were good proxies to explain epidemiological variation in chronotype and sleep duration. Therefore, season of assessment (through DST, photoperiod) and SJl may be confounding factors in epidemiological and genetic studies investigating sleep duration and chronotype differences.

Sleep deprivation, SJl and health

A number of psychiatric disorders have been associated with abnormal sleep and circadian behavior in humans and/or with genes involved in the regulation of these phenotypes in model organisms (for review, see McClung, 2011). In addition, antidepressant and mood-stabilising drugs have been shown to alter the biological clock (treatment with SSRI drugs such as fluoxetine produces a phase advance in the firing of SCN neurons in rat slice culture; for review, see McClung, 2011), suggesting common pathways. The effects of light exposure on brain circuitry and the influence of season on neurobehavioral disorders (for review, see McClung, 2011) resembles the influence of light on the human circadian system (Chang et al., 2011) and may also be linked to gene–environment (G × E) interactions that modulate sleep and circadian behavior. As shown in mice (Ciarleglio et al., 2011), G × E interactions (i.e. imprinting through perinatal photoperiod) tune circadian clock responses to subsequent seasonal photoperiods and hypothetically contribute to the influence of season on neurobehavioral disorders in humans. Circadian misalignment *per se* has been linked to adverse health outcomes, i.e. symptoms of depression (Hasler et al., 2010; Levandovski et al., 2011), metabolic syndrome (i.e. increased glucose and insulin levels; Gonnissen et al., 2012; Roenneberg et al., 2012; Scheer et al., 2009) and obesity (i.e. reduction of leptin, an hormone involved in long term energy balance; Nguyen & Wright, 2009). Investigating the influence of season and DST on human sleep, therefore, is highly relevant

for public health to identify optimal prevention strategies.

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Supplementary material available online

Supplementary Figure S1.

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