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"Early to bed, early to rise": Diffusion tensor imaging identifies chronotype-specificity

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ABSTRACT

Sleep and wakefulness are crucial prerequisites for cognitive efficiency, the disturbances of which severely impact performance and mood as present e.g. after time zone traveling, in shift workers or patients with sleep or affective disorders. Based on their individual disposition to sleep and wakefulness, humans can be categorized as early (EC), late (LC) or intermediate (IC) chronotypes. While ECs tend to wake up early in the morning and find it difficult to remain awake beyond their usual bedtime, LCs go to bed late and have difficulties getting up. Beyond sleep/wake timings, chronotypes show distinct patterns of cognitive performance, gene expression, endocrinology and lifestyle. However, little is known about brain structural characteristics potentially underlying differences. Specifically, white matter (WM) integrity is crucial for intact brain function and has been related to various lifestyle habits, suggesting differences between chronotypes. Hence, the present study draws on Diffusion Tensor Imaging as a powerful tool to non-invasively probe WM architecture in 16 ECs, 23 LCs and 20 ICs. Track-based spatial statistics highlight that LCs were characterized by WM differences in the frontal and temporal lobes, cingulate gyrus and corpus callosum. Results are discussed in terms of findings reporting late chronotypes to exhibit a chronic form of jet lag accompanied with sleep disturbances, vulnerability to depression and higher consumption of nicotine and alcohol. This study has far-reaching implications for health and the economy. Ideally, work schedules should fit in with chronotype-specificity whenever possible.

Introduction

The term 'chronotype' refers to an endogenous, self-sustained disposition towards sleep and wakefulness (Katzenberg et al., 1998; Vink et al., 2001) reflecting preferences in circadian (i.e., oscillation of about 24 h) rhythms. Chronotypes are categorized according to the circadian phase of their biological clock (Kerkhof and Van Dongen, 1996). Specifically, early chronotypes (EC) spontaneously wake up at an early hour and find it difficult to stay up late in the evening. On the other hand, late types (LC) tend to go to bed late at night and sleep late into the day. Chronotype-specificity is promoted by the interplay between neural circadian and homeostatic oscillators (Borbely, 1982): the homeostatic process regulated by adenosine stemming from astrocytes (Halassa et al., 2009) steadily increases when awake and declines during sleep. The circadian rhythm originates in the suprachiasmatic nuclei (SCNs) of the anterior hypothalamus. Chronotype-specificity has been shown to be associated with gender, with a higher percentage of females being ECs (Vink et al., 2001), while high testosterone levels seem to lead to a stronger evening-orientation in young males (Randler et al., 2012). Furthermore, chronotype varies with age (Monk et al., 1997). In particular, teenagers often sleep until late morning and whereas elderly people wake up in the early morning hours. Endocrine factors were reported to be involved in these age-dependent changes of chronotype (Hagenauer et al., 2011). Lastly, the examination of genes contributing to inter-individual differences in sleep architecture, timing, and duration has recently received attention (Lazar et al., 2012).

In particular, LCs show a much larger discrepancy between individual sleep preferences and normal work schedules – typically starting early in the day – that lead to the accumulation of a substantial sleep deficit during the working week as compared to ECs (Roenneberg et al., 2003). Moreover, LCs more frequently report poorer sleep quality, more tiredness during the day (Giannotti et al., 2002; Taillard et al., 2003), exhibit psychological and psychosomatic disturbances (Giannotti et al., 2002), and consume more legal stimulants such as nicotine and alcohol (Adan, 1994; Mecacci and Rocchetti, 1998; Taillard et al., 1999) than ECs. For example, LCs seem to be more vulnerable to bipolar disorders including depression than ECs and intermediate chronotypes (IC, (Mecacci and Rocchetti, 1998; Wood et al., 2009)). Neurostructurally, the white matter (WM) underlying the anterior cingulate gyrus (ACC) and the corpus callosum are reportedly affected by these disorders (Brambilla et al., 2003; Barnea-Goraly et al., 2009). Moreover, the development of depression during the period of a lifetime was shown to be associated with alterations of the white matter underlying the frontal lobes that have

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been linked to specific impairments in cognitive functions (for a review see McKinney and Jacksonville, 2005).

Findings postulated that LCs exhibit a chronic form of functional jet lag (Wittmann et al., 2006) because their endogenous sleep-/wake rhythms rarely fit conventional social schedules. These long-term repeated disturbances of synchronization between the individual, endogenous and the external timing system impair physiological and psychological health and induce stress via high cortisol levels (Winget et al., 1984). Structural changes and cognitive impairments became apparent after five years of exposure to high cortisol levels (Cho, 2001), suggesting that chronic differences in sleep preferences associated with chronotype-specificities may also be associated with brain structural differences. A structural MRI study demonstrated that chronic jet lag produces temporal lobe atrophy leading to spatial cognitive deficits (Cho, 2001). These findings, although stemming from time-zone travelers, highlight the need for structural and not only functional investigations. Consequently, as today's society has become clock driven, so that our sleep/wake behavior is dictated by commercial and industrial demands, the question arises as to whether, for example, chronotype-specificity and health-impairing behavior are associated with specific neural mechanisms.

As brain regions are innervated and connected via white matter bundles, the investigation of WM deserves substantial investigation within the scope of chronotype research. Specifically, WM architecture is crucial for coordinated brain function (for a review see Schmithorst and Yuan, 2010). Various lifestyle habits, e.g. cigarette smoking (for a review see Durazzo et al., 2010), alcohol consumption (Sorg et al., 2012), cannabis use (Zalesky et al., 2012) and sleep deprivation (Rocklage et al., 2009) have been reported to result in WM alterations. As chronotype-specificity determines the individual sleep/wake cycle (Borbely, 1982) and influences lifestyle habits to an enormous extent, the question emerges as to whether it is also associated with the underlying white matter microstructure, especially for LCs who appear to suffer the most from 'chronic jet lag' (Wittmann et al., 2006). Understanding the underlying microstructure by investigating the cerebral WM architecture will lead to consequences for chronotherapeutics (i.e. therapies of phase advanced or delayed sleep disorders) and to the adaptation of work schedules to chronotype-specificities whenever possible.

A powerful tool for the investigation of WM microstructure is Diffusion Tensor Imaging (DTI (Basser et al., 1994)). The commonly used scalar metrics of DTI are mean diffusivity (MD) and fractional anisotropy (FA) that characterize the magnitude and directionality of constrained water diffusion in brain tissue as well as of axial (AD) and radial diffusivity (RD (Song et al., 2003)). AD represents the largest (major) eigenvalue of the diffusion tensor, whereas RD equals the average of two remaining eigenvalues and characterizes diffusivity in the plane orthogonal to the direction of the largest diffusivity. The number of possible valid tracks is known as the so-called fiber count (FC) metric. In particular, the changes in FC were reported with regard to cerebral palsy due to periventricular white matter injury (Thomas et al., 2005). However, it should be mentioned that according to Jones et al. (2013), the term 'streamline count' is sometimes used which offers a more unambiguous way of reporting results. It indicates that there is a dependence on the tractography algorithm and the experimental conditions. It should be pointed out that either "streamline count" or "fiber count" as provided by the tractography tools are not to be confused with a true measurement of the number of actual fibers (i.e. axonal projections). Comparison of cerebral white matter connectivity and architecture between specific groups of subjects was carried out as voxelwise statistical analyses (Ashburner and Friston, 2001). Recently, an algorithm based on track-based spatial statistics (TBSS) was introduced (Smith et al., 2006), which allows for statistical comparisons of multi-subject data at group level using FA and other maps with high reliability due to a simple and clear analysis pipeline.

Based on the reported chronotype-specificities in sleep/wake processes, gene expression and lifestyle habits, the present study aimed at characterizing WM integrity in different chronotypes drawing on DTI metrics such as FA, MD, AD, RD and FC. First, it is hypothesized that LCs differ significantly in their lifestyle habits from ECs and ICs. Second, we question whether significantly different DTI metrics will be present in LCs as they have been reported to be more vulnerable to bipolar disorders, including depression, than ECs and ICs. Hence, the white matter structures underlying the ACC and the corpus callosum are in particular regions of interest as they have been reported to be affected by these disorders. Third, as the development of depression during a person's lifetime is reported to be associated with impairments in cognitive functions, we query whether significant differences will be found in the white matter underlying the frontal lobes. Fourth, based on the previous finding that LCs suffer from chronic functional jet lag, we reveal whether there are differences in the DTI metrics in the white matter underlying the temporal lobes, reported to be affected by chronic jet lag.

Results

Demographic, sleep and lifestyle characteristics

The results of demographic, sleep and lifestyle characteristics are shown in Inline Supplementary Table S1. Statistical differences were revealed for smoking and alcohol consumption with LCs smoking significantly more cigarettes per day and consuming more units of alcoholic beverages per week than ICs ($P < 0.05$). Moreover, LCs reported drinking significantly more alcoholic beverages than ECs ($P < 0.01$). Based on inclusion criteria, no significant differences were detected for age, education, Pittsburgh Sleep Quality Index (PSQI (Buysse et al., 1989)), Epworth Sleepiness Scale (ESS (Chervin, 2003)), Karolinska Sleepiness Scale (KSS (Akerstedt and Gillberg, 1990)) and Beck Depression Inventory (BDI (Kumar et al., 2006)) scores. The reported findings that LCs in particular suffer from 'chronic jet lag' and our findings that they also consume more legal stimulants than ECs and ICs, have led to the combining of ECs and ICs in one group for the DTI metrics analysis.

Inline Supplementary Table S1 can be found online at http://dx.doi.org/10.1016/j.neuroimage.2013.07.086. Q2 Q3

White matter integrity measures

Comparison of early and late chronotypes

In comparison to ECs, LCs showed significantly lower FA values ($P < 0.05$) in the WM underlying the left cingulate and anterior cingulate gyrus as well as in the left frontal lobe (Fig. 1a, S2, S3). ECs on the other hand exhibited a significant decrease of MD values ($P < 0.05$) when compared to LCs, mainly in the left cerebrum, sub-lobar, extranuclear and the left frontal lobe, sub-gyral and in the WM underlying the precentral gyrus (Fig. 1b, S2, S3). AD did not yield significant results.

Comparison of intermediate and late chronotypes

For LCs, significantly ($P < 0.05$) lower FA values emerged in the left corpus callosum (Fig. 2a, S2, S3) and in the WM underlying the left cingulate and anterior cingulate gyri as well as in the left frontal lobe, sub-gyral, as compared to ICs. Additionally, late chronotypes showed lower FC values ($P < 0.05$) for the right frontal lobe, sub-gyral (Figs. 2c, S2, S3). ICs exhibited significantly lower values of RD ($P < 0.05$) compared to the LCs in the left temporal and parietal lobe, sub-gyral (Fig. 2b, S2, S3). AD did not yield significant results.

Comparison of early and intermediate chronotypes

TBSS did not reveal any differences of diffusivity between ECs and ICs.

Comparison of early plus intermediate versus late chronotypes

LCs showed significantly lower FA values ($P < 0.05$) in the WM underlying the left cingulate and anterior cingulate gyrus as compared to the combined EC and IC (EC + IC) chronotypes (Fig. 3a, S2, S3).

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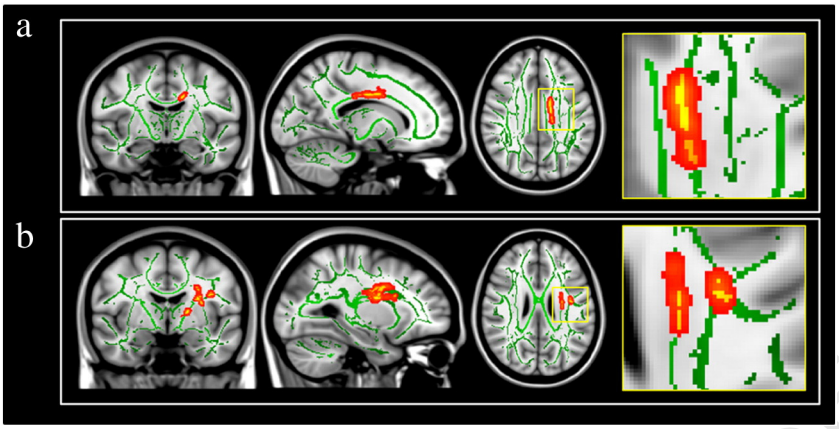

Fig. 1. Significant differences in a) FA and b) MD for ECs compared to LCs. Background: mean track skeleton (green) and T_1-weighted MNI 152 brains. Radiological convention. The right inset represents zoomed view of axial projection marked by a yellow square. a) Coronal, sagittal and axial projections demonstrate regions (red), where FA values are significantly lower for LCs ($P < 0.05$). MNI coordinates of coronal, sagittal, and axial projections are X = −15, Y = −9, Z = 33, respectively, and correspond to center of region. b) Coronal, sagittal and axial projections demonstrate regions (red), where MD values are significantly lower for ECs ($P < 0.05$). MNI coordinates of coronal, sagittal, and axial projections are X = −27, Y = −5, Z = 26, respectively, and correspond to center of region.

Additionally, substantially lower FC values ($P < 0.05$) for the LCs were observed in the right frontal lobe, sub-gyral, (Fig. 3b, S2, S3). MD and RD values were significantly lower for EC + IC (Fig. 3c/d, S2, S3) in the left temporal lobe, sub-gyral ($P < 0.05$). AD did not yield significant results.

Discussion

The present study aimed to characterize the WM architecture of ECs, LCs and ICs by DTI/TBSS. As expected, lifestyle habits differed between chronotypes. More importantly, differences in white matter integrity were prominent, particularly in LCs as compared to ICs and/or ECs. Four specific hypotheses were tested. First, in line with our hypothesis, we identified significant differences between chronotypes, with LCs consuming significantly more nicotine and alcohol than ICs and ECs. Confirming our second hypothesis, significantly different DTI metrics of white matter structures underlying the ACC and the corpus callosum were present. In line with our third hypothesis, significant differences in the white matter underlying the frontal lobes were observed. Confirming our fourth hypothesis, DTI metrics were deviant in the white matter underlying the temporal lobes.

Screening confirmed that numbers of alcoholic drinks per week and number of cigarettes per day in the present study did not qualify for alcohol or nicotine abuse. Nevertheless, while having excluded subjects

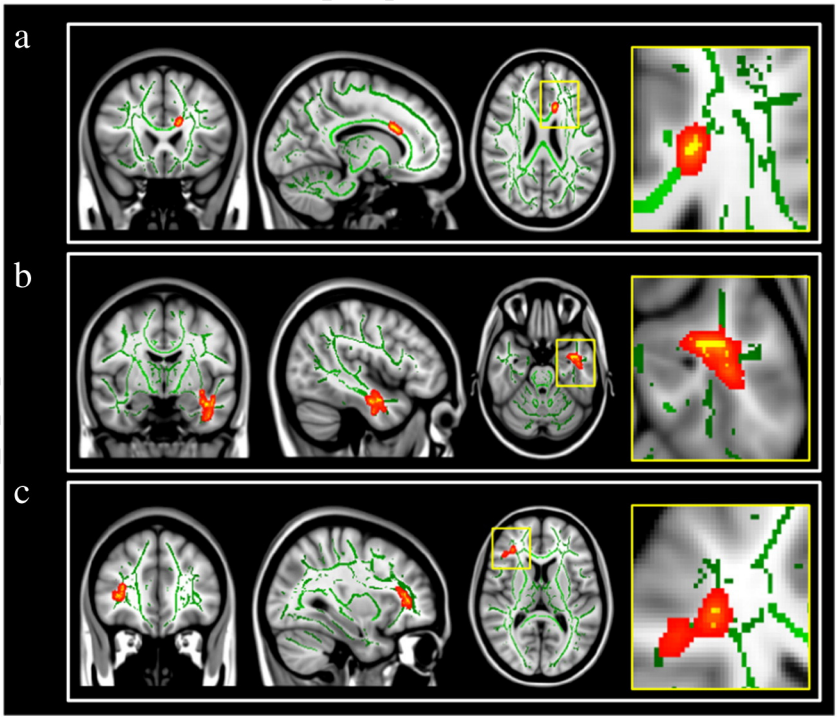

Fig. 2. Significant difference in a) FA, b) RD and c) FC for ICs compared to LCs (IC/LC). Background: mean track skeleton (green) and T_1-weighted MNI 152 brains. Radiological convention. The right inset represents zoomed view of axial projection marked by a yellow square. a) Coronal, sagittal and axial projections demonstrate regions (red), where FA values are significantly lower for LCs ($P < 0.05$). MNI coordinates of coronal, sagittal, and axial projections are X = −13, Y = 22, Z = 24, respectively, and correspond to center of region. b) Coronal, sagittal and axial projections demonstrate regions (red), where RD values are significantly lower for ICs ($P < 0.05$). MNI coordinates of coronal, sagittal, and axial projections are X = −44, Y = −7, Z = −25, respectively. c) Coronal, sagittal and axial projections demonstrate the regions (red), where FC values are significantly lower for LCs ($P < 0.05$). MNI coordinates of coronal, sagittal, and axial projections are X = 32, Y = 36, Z = 10, respectively, and correspond to center of region.

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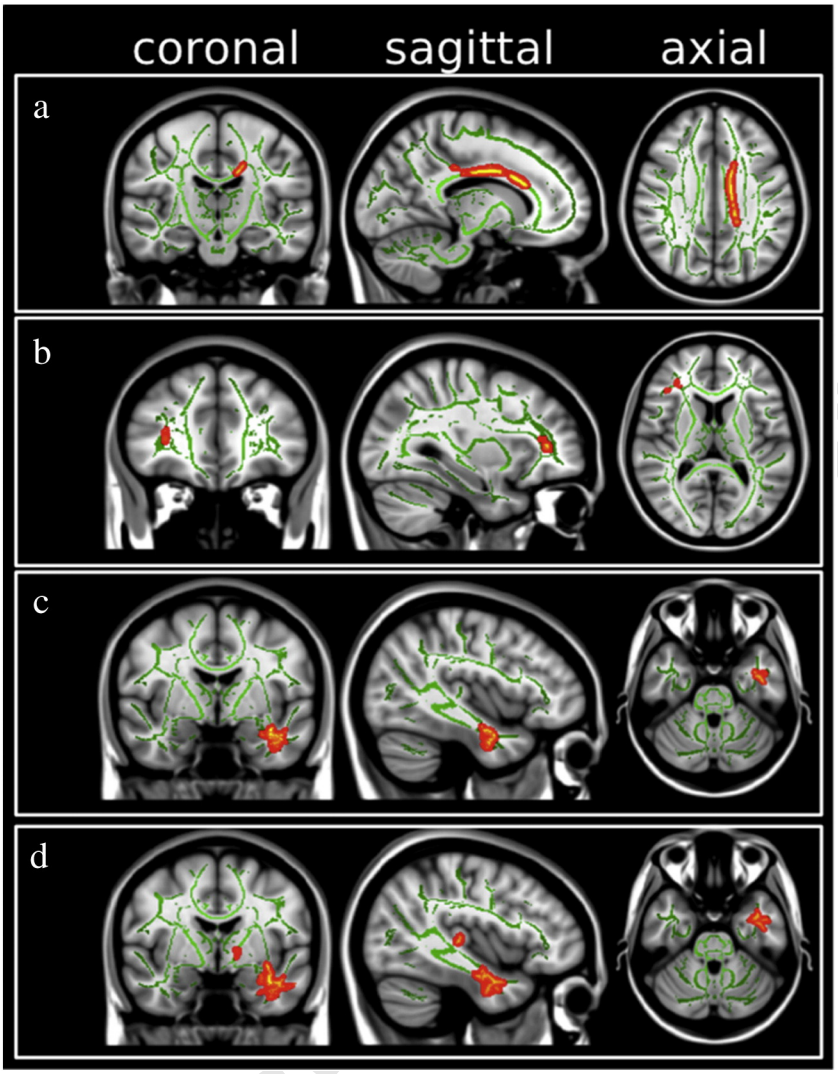

Fig. 3. Significant differences in a) FA, b) FC, c) MD, and d) RD of ECs and ICs as compared to LCs (EC + IC/LC). Background: mean track skeleton (green) and T_1-weighted MNI 152 brains. Radiological convention. a) Coronal, sagittal and axial projections demonstrate the regions (red) where the FA values are significantly lower for LCs ($P < 0.05$). MNI coordinates of coronal, sagittal, and axial projections are X = −15, Y = −9, Z = 33, respectively, and correspond to center of region. b) Coronal, sagittal and axial projections demonstrate the regions (red) where the FC values are significantly lower for LCs ($P < 0.05$). MNI coordinates of coronal, sagittal, and axial projections are X = 32, Y = 36, Z = 10, respectively, and correspond to center of region. c) Coronal, sagittal and axial projections demonstrate the regions (red) where the MD values are significantly lower for the EC + IC group ($P < 0.05$). MNI coordinates of coronal, sagittal, and axial projections are X = −41, Y = −4, Z = −29, respectively. d) Coronal, sagittal and axial projections demonstrate the regions (red) where the RD values are significantly lower for the EC + IC group ($P < 0.05$). MNI coordinates of coronal, sagittal, and axial projections are X = −41, Y = −4, Z = −29, respectively, and correspond to center of region.

with excessive use of nicotine and alcohol, consumption of alcohol and nicotine was more prominent in LCs, thus confirming our first hypothesis. It is well known that LCs consume significantly more alcohol and nicotine in real life (Mecacci and Rocchetti, 1998; Taillard et al., 1999). However, further statistical analyses revealed no significant correlations between alcohol consumption and significant different DTI metrics (see S4) showing that alcohol consumption is not related to our findings. Additionally, alcohol consumption did not account for the significant differences of DTI metrics (see S5). While most of our participants consumed alcohol (see Participants), smokers among the chronotype groups were unequally distributed with only one and two smokers among the ECs and ICs, respectively, but eleven smokers among the LCs. Therefore, correlation analysis was performed only with smoking LCs. Here, smoking was an unrelated factor (see S6 and S7) and could not explain the observed differences in the clusters, thus confirming that it cannot have influenced our findings.

In line with our second hypothesis, LCs exhibited significantly lower FA values in the WM underlying the left ACC as compared to ECs and ICs. Interestingly, DTI studies assessing elderly patients suffering from major depression revealed white matter ACC abnormalities (Alexopoulos et al., 2002; Ballmaier et al., 2004), which affected emotional modulations and cognitive functioning. For the present study, current or past neuropsychiatric disorders were ruled out and subjects were between 18 and 35 years of age, making it difficult to compare results with studies investigating elderly populations. However, LCs were reported to be in particular more vulnerable to develop depression during their lifetime than ICs and ECs (Levandovski et al., 2011) as well as bipolar disorders (Wood et al., 2009). Hallmarks of bipolar disorders are emotional and behavioral disturbances, which are accompanied by episodes of mania and depression and were reported to exhibit cognitive deficits in attention, executive function and short-term memory (Murphy et al., 2001; Altshuler et al., 2005; Pavuluri et al., 2006). In conjunction with chronotype-specific ACC findings, the present study revealed lower FA values underlying the left corpus callosum for LCs compared to ICs. Among others, abnormal corpus callosum integrity was reported to be associated with bipolar disorders (Brambilla et al., 2003; Barnea-Goraly et al., 2009). Thus, future studies probing, e.g. the white matter integrity in LCs suffering from these disorders versus healthy LCs

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might clarify whether our DTI data could serve as a first hint for the development of diseases such as depression, and determine the relationship between chronotype-specificity and the observed white matter alterations underlying the ACC and the corpus callosum. Vice versa, studies focusing on these regions of interest should consider chronotype classification, affective symptoms and cognitive performance as modulating factors.

Confirming our third hypothesis, LCs presented substantially lower FC values in the WM underlying the right frontal lobe compared to ECs and ICs while ECs showed lower MD values for the left frontal lobe compared to LCs. White matter microstructure underlying the frontal lobes has been associated with disturbances in motor movements and cognitive functions, e.g. attention (Kiernan and Hudson, 1994). The interrelation between motor and cognitive skills is well established (see Diamond, 2000, for a review) — with a close interrelation of motor development and cognitive development as well as of the cerebellum and the prefrontal cortex. A circadian modulation of motor skills has already been reported (Drust et al., 2005; Jasper et al., 2011) but no studies focused on chronotype-specificities of motor skills. Given that we did not probe visuomotor coordination or fine motor skills, we cannot comment on potential behavioral effects.

In line with our fourth hypothesis, chronotype-specific DTI metrics were also identified for the WM underlying the left temporal lobe. A structural MRI study assessing WM in time-zone travelers, suffering from physiological and psychological stress, demonstrated that chronic jet lag results in temporal lobe atrophy leading to cognitive deficits in tasks probing spatial functions (Cho, 2001). Current or past psychiatric and neurological diseases and depression served as exclusion criteria for the present study and subjects were young and healthy. Additionally, no significant correlations between a self-rated depression questionnaire (BDI) and significant different DTI metrics clusters emerged (see S4), showing that these potential influencing factors have been successfully ruled out by our screening procedure. Thus, it still has to be determined whether the significant alterations in DTI metrics can be used as an indicator for the development of chronic functional jetlag during aging.

Additional to our four main findings, significantly lower MD values were observed for ECs and in the conjunction of ECs and ICs versus LCs underlying the left precentral gyrus. This region has been linked to disturbances of the somatosensory pathways (Kiernan and Hudson, 1994; Thomalla et al., 2009). Studies investigating chronotype-specificities in somatosensory processing, however, are rare; one potential hint for a particular association between chronotype and somatosensory pathways stems from the fact that LCs are more frequently affected by fibromyalgia syndrome, a diffuse musculoskeletal pain disorder with symptoms of sleep disturbances (Kantermann et al., 2012). As LCs show a significant higher vulnerability to sleep disturbances (Giannotti et al., 2002), a joint physiological, potentially brain structural and functional basis may be underlying. If and in how far our results can be directly linked to fibromyalgia and sleep disturbances can only been clarified by combined studies investigating patient populations of fibromyalgia with DTI under consideration of chronotype and thus investigating the causal roles of late chronotypes and their vulnerability to sleep disturbances and the development of fibromyalgia.

The above findings represent important white matter characteristics underlying chronotype-specificity. The current study design successfully ruled out that modulating factors, e.g. sleep disturbances, affected our results. Although LCs showed higher values of disturbed sleep quality in the PSQI as compared to ICs, this difference was not significant. Moreover, no relationship existed between the self-rated sleep questionnaires and significant DTI metrics (see S4), which supports the notion that none of the subjects suffered from disturbingly increased daytime sleepiness or sleep disorders. In the literature however, LCs were reported to show a larger discrepancy between their preferred sleep time and normal work schedules during their lifetime that led to a substantial sleep deficit during the week as compared to ECs (Giannotti et al., 2002; Taillard et al., 2003). Advisably, chronotype-specificity should be as well be investigated with electroencephalographic (EEG) recordings during sleep and then could be linked to findings of white matter integrity measures to gain insights into group differences of sleep-related pathways.

Several limitations of the present study must be highlighted. First, we only included male subjects to avoid adding another level of statistical complexity and variance. Hence, our results need to be replicated in a sample of females. Based on the present cross-sectional study design, a causal relationship between chronotype-specificity, lifestyle habits and WM structure cannot be delineated. In particular, patient and intervention studies might reveal insights to chronotype-specificity and the associated findings, e.g. higher vulnerability to depression, cognitive impairment and sleep disturbances. Furthermore, the cross-sectional design of the present study allows for the identification of "disturbances of diffusion" in white matter structures in young LCs that have to be further investigated during adulthood and aging in longitudinal studies. As already pointed out above, although our strict exclusion criteria ensured that the subjects did not suffer from psychiatric and neurological diseases, we cannot rule out the possibility of a later development of e.g. depressive disorders. As chronotype-specificity is complex and not fully understood in its neural and genetic depth, it is possible that common genetic factors influence both chronotype classification and WM structure. Consequently, sources and the directionality of influencing factors have still to be determined. There could even be factors involved that have not been considered so far. Moreover, we cannot comment on whether our brain structural findings relate to behavioral or brain functional measures or whether different stress levels affected results. Future studies may want to add neuropsychological testing, brain functional scans or cortisol levels.

The present study is a first successful attempt to shed light on the underlying white matter architecture of chronotype-specificity. Differences of white matter integrity were prominent in LCs as compared to ICs and/or ECs in regions of the frontal, temporal lobes as well as the corpus callosum. Follow-up studies will clarify the link between these white matter alterations in LCs and the potential development of chronic functional jet lag and its associated symptoms during adulthood. The study of chronotype-specificity has far-reaching implications not just for health but also for the economy: ideally, work schedules, especially shift work, should fit in with chronotype-specificity to reduce suboptimal or even erroneous performance at work, which is often associated with higher accidents rates.

Materials and methods

Participants

16 healthy male ECs, 20 ICs and 23 LCs completed the study protocol. Categorization of individual chronotypes was based on the Munich Chronotype Questionnaire (Roenneberg et al., 2003). Subjects were recruited by internet alerts, newsletters and flyers and were financially compensated for participation. Besides common MRI exclusion criteria (e.g. incorporated metal such as retainers, pacemakers, tattoos etc.), study-specific inclusion criteria were (1) age 18–35 and right-handedness according to the Edinburgh Inventory of Handedness (Oldfield, 1971). The following served as exclusion criteria: (a) current or past psychiatric, neurological, or relevant medical disease (e.g. head trauma with unconsciousness >5 min) as determined by experienced raters using a short version of the SCID (Saß and Zaudig, 1996), (b) daily consumption of more than four cups of coffee, caffeinated soft drinks, or energy drinks, (c) history of night work or shift work, (d) crossing of more than two time zones during the last three months prior to the study, (e) symptoms of a possible sleep disorder according to the PSQI (Buysse et al., 1989) or the ESS (Chervin, 2003) and (f) a depressed state according to the BDI (i.e. <8; Kumar et al., 2006). The local Ethics Committee (RWTH Aachen University) approved the study protocol, screening questionnaires and consent forms. All subjects provided

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written informed consent prior to participation. Participants were instructed that the study was aimed to investigate differences in chronotypes and were requested to maintain a regular sleep-wake schedule during the last week prior to admission. Moreover, on the day of the MRI scan, participants were asked not to practice sports, to abstain from alcohol and energy drinks and not to consume coffee/tea or caffeinated beverages for at least 3 h prior to scanning. Immediately prior to the DTI session, each participant's subjective sleepiness was rated on the KSS (Akerstedt and Gillberg, 1990). Among ECs, ICs and LCs, there were a) one, two and eleven smokers, respectively, and b) twelve non-alcohol consumers (five ECs, three ICs and three LCs).

Data acquisition

The MRI session was conducted ten to twelve hours after each individual's wake up time as determined by sleep diaries. Scanning was performed on a 3 T MAGNETOM Tim Trio scanner (Siemens, Erlangen, Germany) using standard gradients and a circular polarized phase array head coil. Participants lay in a supine position; head movement was limited by foam padding within the head coil. Diffusion-weighted data was acquired with the standard double-refocused spin-echo echo-planar imaging using the following parameters: diffusion weightings, *b*-value = 1000 s mm^{-2}, 72 slices, 30 directions, four averages, voxel size of measurement: 1.9 × 1.9 × 1.9 mm^3. For anatomical localization, a magnetization prepared rapid gradient echo (MP-RAGE) sequence was acquired during the imaging session (TR = 2250 ms; TE = 3.03 ms; ST = 1 mm; FOV = 256 × 256 mm; voxel size = 1.0 × 1.0 × 1.0 mm^3).

Data analysis

Data analysis was performed in FSL with FDT tool (Smith et al., 2006). Pair comparisons of FA, MD, AD, and RD maps between EC/IC, EC/LC, IC/LC, and EC + IC/LC groups were performed. Streamline tractography was performed for all groups using the ExploreDTI tool (Leemans et al., 2009). Additionally, the fiber track count maps were exported using ExploreDTI. The fiber track count map takes into account the number of all streamlines that can be reconstructed between two regions of interest for the given voxel. The parameters of deterministic streamline fiber tracking algorithm were the same for all subjects and equal to the following: FA threshold 0.2, angle deviation 30°, minimal and maximal length of fibers 50 and 500 mm, respectively. Determination of the location of voxels demonstrating significantly different DTI values was accomplished by converting the x, y, and z coordinates for the peak voxel within a cluster from the Montreal Neurological Institute (MNI) coordinates used in FSL, to Talairach coordinates (Talairach and Tournoux, 1988) using the MNItoTAL software (http://www.bioimagesuite.org/Mni2Tal/index.html). Demographic data, sleep questionnaires and lifestyle habits were analyzed using One-Way ANOVA with chronotype as between — subject factor (post-hoc test, Bonferroni — corrected). According to Miller and Chapman (2001), it would be inappropriate to include an unequal number of nicotine consumers as a covariate in the correlation analysis. Therefore, correlation analysis was performed only with smoking late chronotypes.

TBSS pipeline in FSL

Data analysis was performed with the FSL toolkit (Woolrich et al., 2009). All datasets were averaged over 4 acquisitions and then corrected for motion/eddy current distortions using the *eddy correct* utility of FSL. Noise level was then estimated and the target images using a background noise correction scheme (Maximov et al., 2012) by in-house Matlab script (The MathWorks, Natick, MA, USA) were corrected. The brain volume was extracted by the application of BET algorithm (Smith, 2002). The FA, MD, AD and RD were calculated using FDT. The workflow of the TBSS approach can be summarized as follows: first, all FA images were preprocessed by being scaled and aligned with each other, removing possible outliers and distortions. After target identification, all FA maps were aligned, transformed, and co-registered by the FNIRT (Andersson et al., 2007) utility of FSL into 1 × 1 × 1 mm^3 Montreal Neurological Institute (MNI 152) space using the FMRIB58_FA image as a template. All other manipulations with images were performed using this space and resolution. Next, mean FA images were prepared and the mean FA skeleton was produced, which represents the center of all possible tracks overall for all groups. In order to exclude gray matter and cerebrospinal fluids, and to avoid high inter-subject variability, the threshold ≥0.2 was applied. Finally, the aligned FA images of each subject were projected onto the mean FA skeleton. Thus, for each subject a personal FA skeleton was produced and then an in-group comparison using the 'randomize' function with 10,000 permutations was calculated. All group comparisons were performed using two-sample unpaired *t*-tests. The TBSS analyses of other maps such as MD, RD, and FC were obtained in the same manner. The statistical threshold $t > 3$, $P < 0.05$, corrected for multiple comparisons with threshold-free cluster enhancement (TFCE) was used for this analysis (Smith and Nichols, 2009).

Whole-brain fiber tracks of LCs, ECs and ICs are provided in S8. S8 represents fiber tracking assessed using a deterministic streamline algorithm for randomly selected volunteers from three groups. The coronal and sagittal projections of whole-brain tractography clearly exhibit an absence of any kind of artifacts and allow one to easily identify multiple anatomical regions with associated fiber tracks. In order to emphasize the small, visually detected variation in fiber tracking between the groups, we presented a well-known fiber track of the corpus callosum.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.neuroimage.2013.07.086.

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