Sleep-wake profiles and circadian rhythms of core temperature and melatonin in young people with affective disorders

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Article info
Article history:
Received 25 January 2017
Received in revised form 5 July 2017
Accepted 7 July 2017

Keywords:
Sleep
Circadian
Melatonin
Core temperature
Affective disorders

Abstract
While disturbances of the sleep-wake cycle are common in people with affective disorders, the characteristics of these disturbances differ greatly between individuals. This heterogeneity is likely to reflect multiple underlying pathophysiologicals, with different perturbations in circadian systems contributing to the variation in sleep-wake cycle disturbances. Such disturbances may be particularly relevant in adolescents and young adults with affective disorders as circadian rhythms undergo considerable change during this key developmental period. This study aimed to identify profiles of sleep-wake disturbance in young people with affective disorders and investigate associations with biological circadian rhythms. Fifty young people with affective disorders and 19 control participants (aged 16–31 years) underwent actigraphy monitoring for approximately two weeks to derive sleep-wake cycle parameters, and completed an in-laboratory assessment including evening dim-light saliva collection for melatonin assay and overnight continuous core body temperature measurement. Cluster analysis based on sleep-wake cycle parameters identified three distinct patient groups, characterised by ‘delayed sleep-wake’, ‘disrupted sleep’, and ‘long sleep’ respectively. The ‘delayed sleep-wake’ group had both delayed melatonin onset and core temperature nadir; whereas the other two cluster groups did not differ from controls on these circadian markers. The three groups did not differ on clinical characteristics. These results provide evidence that only some types of sleep-wake disturbance in young people with affective disorders are associated with fundamental circadian perturbations. Consequently, interventions targeting endogenous circadian rhythms to promote a phase shift may be particularly relevant in youth with affective disorders presenting with delayed sleep-wake cycles.

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1. Introduction
Sleep-wake cycle disturbances are common in affective disorders including difficulty initiating and maintaining sleep (Alvaro et al., 2013; Baglioni et al., 2011; Benca et al., 1992; De Crescenzo et al., 2016; van Mill et al., 2010), abnormal sleep length (Benca et al., 1992; De Crescenzo et al., 2016; Nota et al., 2015; van Mill et al., 2010), and abnormal timing of rest and activity (Nota et al., 2015; Robillard et al., 2015; Salvatore et al., 2008). However, the nature of these disturbances is considerably heterogeneous, with significant variation in the type and severity of sleep-wake abnormalities between individuals (Wulff et al., 2009). Circadian rhythm disturbances have also been found in affective disorders, including abnormalities in circadian rhythms of hormone secretion and core body temperature (Germain and Kupfer, 2008; Gonzalez, 2014; Lange et al., 2012; McIntyre et al., 1990; Melo et al., 2016). Analogous to the sleep-wake cycle findings, there is considerable variation in the nature of reported circadian abnormalities, with several contradictory and inconsistent findings between studies (Germain and Kupfer, 2008; Gonzalez, 2014; Lange et al., 2012; McIntyre et al., 1990; Melo et al., 2016). Characterisation of biological circadian rhythms in relation to specific profiles of sleep-wake cycle disturbance is necessary in order to better understand the

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mechanisms by which different sleep-wake abnormalities can occur, and to identify biological disturbances that may need to be corrected in order to successfully treat sleep-wake cycle disturbances and associated affective symptoms.

In healthy adults, robust circadian rhythms are found in core body temperature and secretion of the pineal hormone melatonin. Core body temperature typically shows a peak in the late afternoon and a trough in the early hours of the morning (Aschoff, 1983), whereas melatonin secretion increases across the evening, peaks during the night, and decreases in the morning (Cajochen et al., 2003). Sleep is normally initiated in the evening, during the rapid decline of core temperature and increase in melatonin secretion (Cajochen et al., 2003; Van Someren, 2006). In those with delayed sleep-wake timing, delays are found in core temperature and melatonin rhythms (Chang et al., 2009; Micic et al., 2015; Saxvig et al., 2013; Uchiyama et al., 2000). Abnormalities in these rhythms have also been found in those with sleep difficulties, with increased nocturnal core temperature (Lack et al., 2008) and decreased melatonin (Hajak et al., 1995; Riemann et al., 2002) in those with sleep maintenance insomnia (increased waking during the night). Further, insomnia with difficulty falling asleep has been associated with a delayed temperature rhythm, whereas insomnia with early morning wake times has been associated with an advanced temperature rhythm (Lack et al., 2008). This is consistent with the notion that there is an optimal circadian time for sleep to occur, characterised by lower core temperature and high melatonin. Accordingly, difficulty initiating or maintaining sleep may occur when sleep is attempted outside of this optimal time or when there is a reduced circadian drive as indicated by decreased amplitude of circadian rhythms.

In individuals with affective disorders, several studies have demonstrated smaller core temperature amplitude, driven by higher nocturnal core temperature, compared to healthy controls (Avery et al., 1982, 1986; Daimon et al., 1992; Souetre et al., 1988, 1989; Tsujimoto et al., 1990; von Zerssen et al., 1985). Some studies have also found decreased amplitude of the melatonin rhythm (Beck-Friis et al., 1984; Fountoulakis et al., 2001; McIntyre et al., 1987; Monteleone et al., 1994; Souetre et al., 1989), however others have found increased melatonin amplitude (McIntyre et al., 1990; Rabe-Jabloniska and Szymańska, 2001; Rubin et al., 1992; Shaffi et al., 1996), null results (Carvalho et al., 2006; Thompson et al., 1988), or more complex results with varied findings between measures (Bumb et al., 2016; Kennedy et al., 1996).

Reports on the timing of these rhythms are similarly inconsistent, with some support for both delayed (Crasson et al., 2004; Monteleone et al., 1994; Robillard et al., 2013) and advanced (Millet et al., 1998; Nair et al., 1984; Novakova et al., 2015) patterns of melatonin secretion, and largely null results for core temperature phase (Avery et al., 1982; Avery et al., 1986; Daimon et al., 1992; Souetre et al., 1988, 1989; Tsujimoto et al., 1990; von Zerssen et al., 1985). However, despite a lack of mean core temperature phase abnormalities, consideration of individual data shows that several patients fall outside the normal range, with reports that between 6 and 21% are phase advanced while 23–24% are phase delayed (Daimon et al., 1992; Tsujimoto et al., 1990). Likewise, there have been reports of abnormally elevated (Bouwman et al., 2014) and abnormally delayed (Robillard et al., 2013) melatonin in some individuals with affective disorders. These different biological circadian abnormalities are likely to lead to different presentations of sleep-wake cycle disturbance, and may account for some of the heterogeneity in sleep-wake profiles in those with affective disorders. There is also some preliminary evidence that delayed circadian rhythms relative to sleep (indicated by phase angles [time differences] between circadian phase markers and sleep) are associated with increased depressive symptom severity in affective disorders (Emens et al., 2009; Hasler et al., 2010), which may indicate misalignment between circadian signals and rest-activity rhythms in those with more severe illness.

Across adolescence and young adulthood, sleep-wake cycles and biological rhythms undergo substantial changes (Carpen ter et al., 2015a; Gradisar et al., 2011). There is also a heightened incidence of affective disorders across this period (Burke et al., 1990; Merikangas et al., 2010), and thus the potential influence of circadian factors in the development of these disorders may be particularly relevant. Although delayed sleep timing, short sleep duration, and insomnia are associated with increased risk of affective disorders in adolescence (Blank et al., 2015; Sivertsen et al., 2014; Zhang et al., 2016), research exploring the role of circadian rhythms in this relationship is limited. In an initial study characterising circadian rhythms in young people with affective disorders we have shown that those at later stages of illness present with reduced melatonin secretion across the evening and shorter phase angles between dim light melatonin onset (DLMO) and sleep onset (Naimsh et al., 2012). However, we have failed to find any linear associations between symptom severity and dim light melatonin measures in this population (Carpenter et al., 2016). In contrast, recent research in young psychiatric outpatients has found that low melatonin levels are associated with increased depressive symptoms independent of diagnosis (Sandberg et al., 2016). Sleep-wake abnormalities have been highlighted as potential transdiagnostic factors in psychiatric disorders (Benca et al., 1992; Dolsen et al., 2014; Harvey et al., 2011), and, in line with the National Institute of Mental Health’s Research Domain Criteria principles (Insel et al., 2010), investigation of sleep-wake abnormalities and their underlying pathophysiology independently of specific diagnoses is warranted. We have also recently shown evidence of distinct subtypes of objective sleep-wake function in young people with affective disorders that are not linked to specific diagnoses (Carpenter et al., 2015b), however the underlying biology of these different sleep-wake profiles is yet to be investigated.

This study aimed to empirically derive subgroups of young people with affective disorders based on objective sleep-wake cycle profiles (as measured by actigraphy), and characterise dim light evening melatonin and overnight core temperature in these subgroups. Cluster analysis was used to identify sleep-wake profiles in individuals with affective disorders for comparison with healthy controls across endogenous circadian parameters. In line with previous research suggesting that phase delay and more disturbed sleep are particularly prominent in young people with affective disorders (Blank et al., 2015; Carpenter et al., 2015b; Robillard et al., 2014; Sivertsen et al., 2014; Zhang et al., 2016), and that reduced amplitudes of core temperature and melatonin are associated with insomnia (Hajak et al., 1995; Lack et al., 2008; Riemann et al., 2002), we hypothesised that profiles defined by delayed sleep would be associated with delayed endogenous rhythms, and profiles defined by disrupted sleep would be associated with lower evening melatonin and higher nocturnal core temperature.

2. Material and methods

2.1. Participants

Fifty young people presenting for outpatient clinical care with affective (anxiety and/or mood) disorders and 19 healthy control participants (all aged between 16 and 31 years) took part in this study as part of a larger ongoing observational study on mental health in youth. Assessment by a mental health professional to determine primary diagnoses indicated that the majority of outpatients presented with depressive (n = 29) or mixed depressive and anxious (n = 16) syndromes, however some were assigned a
bipolar (n = 3) or primary anxiety (n = 2) diagnosis. Exclusion criteria were history of neurological disease (e.g. epilepsy), medical illness known to impact brain function (e.g. cancer), electroconvulsive therapy in the last 3 months, intellectual and/or developmental disability, insufficient English language skills to comprehend the protocol, current substance dependence, and non-24-h sleep-wake disorder (as evidenced by actigraphy patterns). This study was approved by the University of Sydney Human Research Ethics Committee and all participants gave written informed consent.

At the time of assessment, twenty-four participants with affective disorders (48%) were taking psychotropic medications (17 were taking one class of medication, 6 were taking 2 classes). Thirteen (26%) were taking SSRIs, 7 (14%) were taking SNRIs, 7 (14%) were taking mood stabilisers, 1 (2%) was taking an antipsychotic, and 1 (2%) was taking a stimulant.

2.2. Clinical assessment

For the participants with affective disorders, the Hamilton Depression Rating Scale (HDRS), and the Social and Occupational Functioning Assessment Scale (SOFAS) were rated in semi-structured interviews by a psychiatrist or research psychologist. Three participants were missing HDRS data, 3 were missing SOFAS data, and 3 were missing both. Analyses were performed using the HDRS total scores minus the three insomnia items (HDRS-Ins) in order to measure depressive symptomology as distinct from sleep disturbances.

2.3. Sleep-wake assessment

All participants were provided with temperature controlled bedding and were asleep in their beds from 20:00 hours to 07:00 hours, with actigraphy recording device (Actiwatch-64/L/2, Philips Respironics, Pittsburgh, USA or GENEActiv, Activinsights, Kimbolton, UK) for between 5 and 19 days (median 14 days). All sets of data were visually inspected by trained technicians to adjust the start and end of each sleep episode based on the respective drop and rise in activity associated with sleep and wake. Additional information was used to inform these adjustments where available (i.e. sleep diaries, event markers on the device presssed at bed and rise times, and/or drops and rises in ambient light from sensors on some devices). Standard algorithms for Actiwatch data (Oakley, 1997) and equivalent algorithms for GENEActiv data (te Lindert and Van Someren, 2013) were used to identify periods of wake during the sleep episode with a medium sensitivity threshold of 40 counts per epoch. Sleep onset time, sleep offset time, sleep midpoint, wake after sleep onset (WASO: number of minutes during the sleep period scored as wake), and total sleep time (TST: number of minutes during the sleep period scored as sleep) were averaged over the monitoring period.

2.4. Circadian assessment

Within 1 month of the sleep-wake assessment (median 3 days, range 0–27 days), participants attended the Chronobiology and Sleep Laboratory at the Brain and Mind Centre. On the day of circadian assessment, all participants were awake and alert from 07:00 hours to 20:00 hours. The assessment began approximately 8 h prior to participants’ habitual sleep time (HST, i.e. average sleep onset time across the actigraphy recording period) and they were kept in dim-light (<30 lux) while remaining seated as much as possible until approximately 2 h after HST, at which point participants could be moved to sleep until their habitual wake time (HWT, i.e. average sleep offset time across the actigraphy recording period). Saliva samples were collected with Salivette tubes (Sarstedt, Nümbrecht, Germany) every 30 min beginning approximately 6 h prior to HST and concluding approximately 2 h after HST. An ingestible temperature sensor (Equivil LifeMonitor, Equivil, Cambridge, UK) was used to record core temperature from shortly after arrival at the laboratory until approximately 3 h after HWT. Participants were provided with temperature controlled snacks and drinks to minimise disruption of the temperature recording.

Saliva melatonin (200 µl) was assayed in duplicate by double antibody radioimmunoassay (Buhlmann Laboratories AG, Schwanenbuch, Switzerland) with a detection threshold of 0.999 pg/mL (inter-assay coefficient of variation between 8.2% and 15%, intra-assay coefficient: <10.0% across the standard curve). DLMO was defined by linear interpolation based on the two samples surrounding the sample where salivary melatonin concentration reached a threshold of 3 pg/mL and remained above this threshold for the three subsequent samples. The phase angle between sleep and melatonin onset (DLMO-SleepPA) was calculated by subtracting the time of DLMO from sleep onset. Melatonin area under the curve (AUC) was calculated for each participant using the trapezoid method using samples between 4 h prior to HST until 1.5 h after HST. Maximum evening melatonin was defined as the highest melatonin concentration in the samples from 4 h prior to HST until 1.5 h after HST. Six participants (2 with affective disorders and 4 with affective disorders) were excluded from AUC and maximum evening melatonin calculations due to missing samples. Temperature recordings were taken several times per minute and averaged to 1 min epochs across the recording period. The first hour of recording was discarded to remove the initial variation related to gastrointestinal transit. Any epoch in which temperature differed by more than 0.15 °C from the previous minute was considered a non-physiological change in temperature and was discarded along with the following 30 min in which temperature returned to normal. Curve fitting was performed using GraphPad Prism (GraphPad Software, La Jolla, USA) to fit a sine wave with an absolute frequency of <0.5 (no more than one cycle per 12 h) to each participant’s temperature recording. From the best curve fit the core temperature minimum (TempMin; lowest temperature) and nadir (TempNadir; time at which the minimum occurred) were estimated. Curve fitting could not be performed in 6 participants with affective disorders and 3 controls due to insufficient data where recordings terminated early. The phase angle between sleep and temperature (Temp-SleepPA) was calculated by subtracting the time of sleep midpoint from TempNadir.

2.5. Statistical analysis

The Statistical Package for Social Sciences (SPSS for Windows 22.0: SPSS, Inc., Chicago, IL, USA) was used to conduct analyses. T-tests and Chi-squared tests were used to compare patients to controls on age and sex distributions. T-tests or one-way analysis of variance (ANOVA-controlling for significant age or sex differences) were used to compare patients to controls across sleep-wake and circadian variables. K-means cluster analysis was performed in the affective group using the variables sleep onset, sleep offset, TST, and WASO. The number of clusters was specified as 3 based on previous clustering in this population (18 of the affective participants in the current study were included in this previous study) (Carpenter et al., 2015b). Variables were standardised using z-scores and cases were sorted by a random factor prior to clustering. One-way analysis of variance (ANOVA) was then used to characterise these groups in comparison to the control group across sleep-wake measures. One-way ANOVA and Chi-squared tests or Fisher’s exact tests were performed across cluster groups and controls to examine any differences on demographic and clinical measures. Circadian variables were then compared between controls and
cluster groups using one-way ANOVA or ANCOVA (controlling for significant age or sex differences) with Bonferroni-corrected pairwise comparisons. A significance value of p < 0.05 was used for all analyses.

3. Results

Melatonin levels did not reach the DLMO threshold in 11 participants with affective disorders. For two participants with affective disorders the core temperature data did not converge with the sine wave model thus a curve fit was not produced. These cases were excluded from comparisons involving DLMO and core temperature respectively.

Participants with affective disorders and controls did not significantly differ on sex distribution ($\chi^2(1) = 0.50$, p = 0.48), but did differ in age ($t_{67} = 3.91$, p < 0.001), that such controls were significantly older than those with affective disorders. Compared to controls (and controlling for age), those with affective disorders had significantly later sleep offset ($F_{(1,66)} = 8.33$, p = 0.005), later sleep midpoint ($F_{(1,66)} = 5.12$, p = 0.027), later DLMO ($F_{(1,55)} = 4.52$, p = 0.038), and greater WASO ($F_{(1,66)} = 18.01$, p < 0.001), but did not differ in sleep onset, TST, melatonin AUC, maximum evening melatonin, DLMO-SleepPA, TempMin, TempNadir, or Temp-SleepPA (all p > 0.05).

Cluster analysis identified three distinct groups of relatively similar size: one characterised by late sleep onset and offset, and increased WASO relative to controls (delayed sleep-wake cluster, n = 17); one characterised by increased WASO relative to controls (disrupted sleep cluster, n = 18); and one characterised by later sleep offset and long TST relative to controls (long sleep cluster, n = 15).

Mean values for demographic, clinical, sleep-wake, and circadian variables across cluster groups and controls are reported in Table 1. There were significant differences in age ($F_{(3,65)} = 5.49$, p = 0.002) with the control group being significantly older than the disrupted sleep cluster and long sleep cluster. No significant differences were found on sex distribution, HDRS, or SOFAS. Significant differences were found across cluster groups in the number of participants taking medication ($\chi^2(2) = 6.97$, p = 0.031), with a smaller proportion of the disrupted sleep cluster being medication free. There were no differences between groups for specific classes of medication (all p > 0.05). The proportion of participants in which melatonin levels did not reach the DLMO threshold significantly differed between groups (Fisher’s Exact Test, p = 0.018) with a greater proportion in all cluster groups compared to controls.

Controlling for age, there were significant group differences in DLMO ($F_{(3,53)} = 3.98$, p = 0.012) and TempNadir ($F_{(3,53)} = 5.36$, p = 0.003), with later DLMO in the delayed sleep-wake cluster relative to controls, and later TempNadir in the delayed sleep-wake cluster relative to controls and the disrupted sleep cluster. There were no significant differences between cluster groups and controls in maximum evening melatonin, melatonin AUC, DLMO-SleepPA, TempMin, or Temp-SleepPA. Fig. 1 shows sleep, temperature, and DLMO timing in controls and cluster groups.

4. Discussion

This study reported fundamental biological characteristics of the circadian system (i.e. evening melatonin and overnight core temperature) in young people with affective disorders grouped according to objectively measured sleep-wake profiles. The affective disorder group as a whole presented with later DLMO, later sleep offset times, and increased WASO, but did not differ from controls on core temperature nadir, TST, sleep onset times, or phase angles between circadian markers and sleep. Cluster analysis of young people with affective disorders found three subgroups defined by different sleep-wake abnormalities, with the group defined by delayed sleep-wake profiles being the only group to show significantly later circadian parameters (i.e. delayed core temperature and melatonin onset) relative to the control group. The results suggest that distinct sleep-wake profiles exist in young people with affective disorders, and that abnormalities in core temperature and melatonin rhythms accompany delayed sleep-wake cycles, but not other sleep-wake abnormalities.

Three distinct sleep-wake profiles were found in the present sample of young people with affective disorders, and these closely replicate those produced in a previous analysis of participants drawn from the same clinical population (with a partially overlapping sample of 18 participants) (Carpenter et al., 2015b). A

### Table 1

| N | Age (years) | Sex (F/M) | HDRS | SOFAS | Sleep Onset | Sleep Offset | Sleep Midpoint | WASO (min) | TST (min) | No DLMO (n) | DLMO | Melatonin AUC (pg/ml) | Evening Mel | 1. Delayed sleep-wake cluster | 2. Disrupted sleep cluster | 3. Long sleep cluster | Test statistics | Significant pairwise comparisons |
| 19 | 24.5 ± 3.4 | 10/9 | – | – | 24.2 ± 0.9 | 8.2 ± 1.1 | 4.2 ± 0.9 | 47.1 ± 11.8 | 435.8 ± 49.2 | 0 (0) | 22.1 ± 1.9 | 33.8 ± 19.9 | 16.2 ± 9.9 |
| 17 | 21.8 ± 3.0 | 10/7 | 12.9 ± 5.6 | 64.1 ± 8.3 | 26.3 ± 0.8 | 11.1 ± 0.8 | 6.7 ± 0.7 | 80.7 ± 24.7 | 448 ± 45.4 | 5 (29) | 24.0 ± 1.1 | 29.2 ± 27.4 | 13.3 ± 10.6 |
| 18 | 20.8 ± 3.7 | 10/8 | 13.6 ± 6.0 | 63.7 ± 9.6 | 23.6 ± 0.8 | 0.8 ± 0.8 | 3.8 ± 0.7 | 86.1 ± 26.5 | 418.9 ± 37.5 | 5 (28) | 22.3 ± 1.4 | 19.7 ± 15.8 | 10.6 ± 8.7 |
| 15 | 20.6 ± 2.7 | 11/4 | 9.9 ± 4.7 | 69.5 ± 10.0 | 24.2 ± 1.0 | 9.2 ± 0.6 | 4.7 ± 0.7 | 56.1 ± 27.5 | 483.6 ± 41.4 | 1 (7) | 23.0 ± 1.5 | 34.6 ± 42.8 | 18.0 ± 13.2 |

*Test statistics report Chi squared tests, Fisher’s exact test, ANOVA, or ANCOVA (controlling for age-used for circadian comparisons); Sleep onset, offset, midpoint, DLMO, TempNadir, DLMO-SleepPA and Temp-SleepPA are time values in decimal form; HDRS = Hamilton Depression Rating Scale (not including insomnia items); SOFAS = Social and Occupational Functioning Scale; WASO = wake after sleep onset; TST = total sleep time; DLMO = dim light melatonin onset; AUC = area under curve; Mel = melatonin; PA = phase angle; FET = Fisher’s exact test; Ctl = controls.*
greater proportion of participants in the disrupted sleep cluster were taking psychotropic medication, but there were no differences between clusters in the proportion taking specific classes of medication. Importantly, the cluster groups did not differ on severity of depressive symptoms or social and occupational functioning, suggesting that none of the sleep-wake profiles found here are differentiated by more severe or disabling affective illness.

Delayed core temperature nadir and delayed onset of melatonin secretion were found in the cluster with delayed sleep-wake cycles. While previous research has established an increased incidence of affective disorders in young people with delayed sleep-wake timing (Zhang et al., 2016) the present study builds on this to suggest that circadian rhythm delays accompany the sleep-wake cycle delays in this population. This is in line with our hypotheses, and with previous research finding delays in core temperature and melatonin rhythms in some individuals with affective disorders (Crasson et al., 2004; Daimon et al., 1992; Monteleone et al., 1994; Robillard et al., 2013; Tsujimoto et al., 1990). However, other previous research has produced conflicting reports regarding the timing of circadian rhythms in affective disorders, with reports of both delays and advances of melatonin rhythms (Crasson et al., 2004; Millet et al., 1998; Monteleone et al., 1994; Nair et al., 1984; Novakova et al., 2015; Robillard et al., 2013) and no mean phase abnormalities in core temperature rhythms (Avery et al., 1982, 1986; Daimon et al., 1992; Souetre et al., 1988, 1989; Tsujimoto et al., 1990; von Zerssen et al., 1985). The current findings provide a potential explanation for the discrepancy in previous results by suggesting that circadian phase abnormalities may only be present in a subgroup of individuals with affective disorders and not others. Previous samples may have included different proportions of these subgroups, producing different results at the group level. Future research should investigate potential factors contributing to different circadian profiles in these subgroups such as individual differences in exposure to light or other external circadian synchronisers, sensitivity of the circadian system to these synchronisers, or endogenous circadian period. The present results highlight the importance of identifying meaningful subgroups in heterogeneous populations such as these, and identifying factors that may contribute to individual differences. The findings of this study are consistent with the notion that an endogenous delay of circadian rhythms may drive, or serve to maintain, the delayed sleep-wake cycle found in some individuals with affective disorders. Conversely, behavioural delays of sleep-wake timing may lead to a shift in the internal pacemaker and corresponding delays in biological rhythms. The delayed rhythms observed here in a subgroup of young people with affective disorders are analogous to the delays in both sleep and circadian rhythms reported in psychiatrically healthy individuals with delayed sleep phase disorder (Chang et al., 2009; Micic et al., 2015; Saxvig et al., 2013; Uchiyama et al., 2000). Consequently, treatments that shift endogenous rhythms to an earlier phase (such as phototherapy (Al-Karawi and Jubair, 2016) or melatonin based medications (Taylor et al., 2014)), or behavioural interventions to shift sleep-wake timing to an earlier phase may be particularly relevant in these young people with affective disorders to correct the biological and behavioural delays.

Abnormalities in phase angles between melatonin and sleep or core temperature and sleep were not found in those with affective disorders as a group or in any cluster subgroups. Some previous research has found an association between phase angles and depression severity (Emens et al., 2009; Hasler et al., 2010), however we failed to find any linear associations between these factors.
in a previous study of this population (Carpenter et al., 2016) or in Supplementary analyses of the current sample. On the other hand, we have previously found shorter phase angles between DLMO and habitual sleep onset in younger people at later clinical stages of affective illness compared to those at earlier stages (Naismith et al., 2012), suggesting that circadian disruptions may be more apparent in those with more established affective disorders. In addition, the present cluster groups were defined by sleep-wake parameters rather than biological circadian measures, and it remains possible that abnormalities in phase angles are related to depressive symptom severity in some individuals but are not related to the sleep-wake profiles identified here. Future research is needed to further investigate circadian phase angle abnormalities in young people with affective disorders to establish how such abnormalities may relate to both symptoms and course of illness, with longitudinal investigations of particular importance in explaining the contrasting findings.

Disrupted (i.e. more fragmented) sleep was found in both the delayed sleep-wake cluster and the disrupted sleep cluster, however, neither group showed differences in core temperature minimum or evening melatonin levels. This is contrary to our hypothesis, and suggests that the disturbed sleep observed in some young people with affective disorders is overcome by abnormal levels of evening melatonin or abnormal drops in nocturnal core temperature. The absence of any differences in core temperature minimum is in contrast to previous research in affective disorders finding increased nocturnal core temperature (Avery et al., 1982, 1986; Daimon et al., 1992; Souetre et al., 1988, 1989; von Zerssen et al., 1985). A possible explanation for this discrepancy is that previous studies were all done in adult samples, whereas the present sample included young adults and adolescents. It is conceivable that reductions in amplitude of core temperature may arise due to a weakened circadian pacemaker following long-term disturbances in sleep. In this case the associated elevation of nocturnal core temperature would be observed in older and more chronically ill individuals, but not necessarily in younger individuals such as the present sample.

Although this study did not find differences in evening melatonin levels, this does not discount the possibility that those with affective disorders have abnormalities in amplitude of the melatonin rhythm. Indeed, several studies measuring melatonin across a 24 h period have found differences between those with affective disorders and controls only at time-points in the early hours of the morning (when melatonin is at its maximum), and not earlier in the evening (McIntyre et al., 1990; Rabe-Jabłońska and Szymańska, 2001; Shafii et al., 1996). Additionally, the level of melatonin secretion around habitual sleep onset may be influenced by the relative timing of the melatonin rhythm. Melatonin maximum and AUC in the present study were significantly correlated with melatonin-sleep phase angles (see Supplementary analyses) which may have contributed to the lack of significant melatonin secretion differences between groups. Further investigation into 24 h melatonin profiles is necessary to clarify whether melatonin amplitude is altered in subgroups of those with affective disorders.

In the present study, around 20% of those with affective disorders had melatonin levels that did not reach the threshold for DLMO. Previous research in healthy participants and in those with delayed sleep phase disorder has found low melatonin secretion in some individuals (reported prevalence ranging from 3 to 12%) (Burgess and Fogg, 2008; Burgess et al., 2015; Keijzer et al., 2011). This low melatonin secretion may account for some of the cases in which DLMO was not observed in this study, while other possible causes include melatonin onset outside the sampling period, or otherwise abnormal or unstable melatonin production (Bouwmans et al., 2014; Burgess and Fogg, 2008; Leibenluft et al., 1996; Sundberg et al., 2016). The proportion of participants with no detectable DLMO did not differ between cluster groups so there does not appear to be an association with the specific sleep-wake profiles identified here. While it is possible that an abnormal melatonin rhythm contributed to disturbances in sleep in some of these individuals, the cause of undetectable DLMO cannot be conclusively determined and further investigation of the potential causes and consequences of low melatonin around habitual sleep time in affective disorders is warranted.

This study is limited by its cross-sectional design which does not allow us to determine directions of causality. Measures of melatonin and temperature were taken over a limited time period and thus do not provide as much information as complete 24 h assessments, particularly in terms of estimating the amplitude of these rhythms. Notably, measures of melatonin maximum and AUC may not accurately reflect the overall amplitude as abnormalities in the timing of the melatonin rhythm relative to sleep (i.e. phase angles) may also contribute to the amount of melatonin secreted around the time of habitual sleep. It should also be noted that the recording of core body temperature in this study reflects partial masking of the endogenous rhythm by sleep and associated postural changes. The interval between the sleep-wake and circadian assessments was up to 27 days in some participants, which may limit comparability with assessments that are completed within a shorter time interval. Nevertheless, the results of this study provide evidence that distinct profiles of sleep-wake behaviour exist in young people with affective disorders, and suggest that delays in biological circadian rhythms accompany delays in sleep-wake cycles. Future longitudinal research should explore whether delays in circadian rhythms precede or follow sleep-wake cycle delays, how different sleep-wake profiles affect responsiveness to treatments targeted at correcting abnormal circadian rhythms, and how correction of these abnormalities may contribute to improvements in symptoms and functioning in young people with affective disorders.

Conflict of interest

Dr Hermens has received honoraria for educational seminars from Janssen-Cilag and Eli Lilly. Prof. Hickie is the Co-Director, Health and Policy, of the Brain and Mind Centre, which operates two early-intervention youth services under contract to headspace. He is a member of the new Australian National Mental Health commission and was previously the CEO of beyondblue: the national depression initiative, and a director of headspace: the national youth mental health foundation. He has led a range of community-based and pharmaceutical industry-supported depression awareness and education and training programs. He has led depression and other mental health research projects that have been supported by a variety of pharmaceutical partners. Current investigator-initiated studies are supported by Servier and Pfizer. He has received honoraria for his contributions to professional educational seminars supported by the pharmaceutical industry (including Servier, Pfizer, AstraZeneca, and Eli Lilly). All other co-authors have no financial disclosure or conflict of interest to report.

Funding

Prof. Hickie was funded by a National Health and Medical Research Council Program Grant (No. 566529) and Australian Fellowship (No. 464514). Dr Robillard received a postdoctoral training award from the Fonds de la recherche en santé du Québec. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.


